Overcoming yield limitation of canola by improving water use efficiency

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Table of Contents

Abstract

Improved adaptation of canola by breeding has pushed its production into low rainfall areas in southern Australian where previously mustard has been considered a more suitable oilseed. Canola has a high nitrogen (N) requirement and how best to manage N in an environment where rainfall is variable is a challenging problem. Limited research has been undertaken in Australia to look at ways to improve water use efficiency (WUE) and to understand influences of interactions between N, water and seasonal variation on canola seed yield and nitrogen use efficiency (NUE_{GY}).

Field experiments were conducted at a medium rainfall site (Roseworthy) in South Australia between 2011and 2013. These three years experienced contrasting amounts and patterns of rainfall. Different N management strategies in canola and mustard were tested to match the demand and supply for N in each year and in one experiment supplementary irrigation was also used. Two mustard and four canola cultivars, including two triazine tolerant (TT) and two non-TT cultivars, were evaluated under different N application strategies comprising three N rates (0, 100 and 200 kg N ha^{-1}) and different timings of application. A non-limiting control was used in which 200 kg N ha^{-1} was applied in up to five split applications throughout the growing season. Treatments were selected to alter the crop canopy and to assess the balance between N and water use.

Mustard and canola perform similarly in the high rainfall year but canola out-yielded mustard in the season with below-average rainfall. Seed yields of canola and mustard were closely associated with total dry matter production and harvest index (HI) varied little between treatments. Applying N at the rosette stage was the key for achieving high seed yield of canola and mustard as it achieved 85% and 94% of the seed yield obtained with the non-limiting N treatment. Nitrogen rate and timing did not influence total water use of canola and mustard but influenced its partitioning between pre- and post-flowering periods. Nitrogen rate increased water extraction depth at flowering but at maturity all treatments extracted water from a similar depth of soil. Irrigation improved total shoot dry matter by 41% and yield by 49% with a little change in HI. The additional water from irrigation was used almost twice as efficiently as the seasonal water use. Irrigation improved NUE_{GY} but higher N rates decreased NUE_{GY}.

Optimising the sink capacity by improving pre-flowering biomass has an important influence on seed yield of canola and mustard. By delaying and targeting a specific growth stage for N application there was only slight improvement in HI and slight reduction in oil content. Low NUE_{GY} in these environments was mainly related to limitation of low agronomic efficiency and low nitrogen harvest index N uptake and low N uptake efficiency rather than physiological N efficiency.

The study also provides empirical evidence that yields of canola and mustard are co-limited by water and N under the post-sowing N management strategies Analysis of water and N co-limitation found that N was the bigger limiting factor than water. The rate of N rather its timing was found to be important to yield and WUE. This study also indicates that better use of subsoil moisture may be an avenue for improvements in yield and WUE of canola in this environment. Future studies should focus on the interaction of pre and post-flowering water use and targeted N application on rosette stage in devising improved management tools.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Amritbir Singh Riar and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Amritbir Singh Riar. **Date**...23/06/2015...

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Chapter 1

1. General Introduction

Canola belongs to Brassicaceae family along with mustard, turnip, wild radish, cauliflower, broccoli and cabbage. Initially canola was known as rapeseed (McCaffery 2009), or oilseed brassica. Canola is a trademark, which was originally registered by ‘Western Canadian Crusher Association’ and now belongs to Canola Council of Canada. Canola oil is known for its flavouring and medicinal properties (Gunstone 2004). The presence of high erucic acid and glucosinolates in traditional oilseed brassica distinguish it from Canola. Canola has the low erucic acid and glucosinolates which was achieved by the Canadian breeders by natural breeding in the late 1960s and became known worldwide as ‘double zero’ or ‘double low’ varieties. In the past 40 years, these characters became standard for oilseed brassica and words canola and rapeseed became used synonymously. The English speaking countries prefer the word canola whereas non-English speaking countries still use rapeseed (Gunstone 2004).

The uses of rapeseed were recorded in India as early as 2000BC and in Europe in the 13th century. Oilseed rape was grown in Canada in 1942 for industrial use commercial purpose and in 1956 for edible purpose (McCaffery 2009). Since the introduction of canola to Australia in late 1960s, canola has developed into an important broadleaf rotational crop and also providing national economic benefits in employment, export and emerging as a major oilseed industry (McCaffery 2009).

Canola is the third most important crop of Australia, in terms of area occupied, after wheat and barley. Australia produces around 1.4 million tonnes of canola per annum (McCaffery 2009). Australia has a significant international market for canola. Prior to the 2003 drought, Australia was exporting 1.3 million tonnes of canola regularly (AOF 2010).

Mustard (*Brassica juncea*) is an alternative oilseed for water limited environments. Studies in Australia have shown better adaptation of mustard than canola in low rainfall areas (Wright *et al.* 1995; Hocking *et al.* 1997a; Gunasekera *et al.* 2009) due to its ability to flower and ripen more rapidly in water stress conditions. The widespread cultivation of mustard and rapeseed in India during the dry season shows their ability to adapt to the water-limited environments. In India rapeseed and mustard are cultivated on stored soil moisture with water stress increasing as the season progresses: under these conditions yields range between 524-1559 kg ha^{-1} (Kumar and Chauhan 2005), which is similar to canola grown in Australia. Efforts have been made by the breeders and agronomists in recent years to narrow the yield gap between optimal and water deficits conditions (Cattivelli *et al.* 2008) but with little success so far.

The perception of canola as a high input and high risk crop with inconsistent quality has limited expansion of area under canola in Australia. Nitrogen is one of the most important and expensive inputs for canola production. Application of N significantly increases plant height, dry matter, number of primary and secondary branches per plant at harvesting, siliqua per plant and yield (Singh and Meena 2004). However, the efficiency of N use in cropping is low, generally remaining between 30-50% (Fageria and Baligar 2005). Due to environmental issues such as pollution of ground water table and rising cost of production, achieving higher yields with greater N use efficiency have become a challenge for agriculture. These goals cannot be achieved without improving the understanding of physiological processes involved in biomass production and yield of canola. A mismatch between the biomass production and harvest index of canola is an important issue in canola production. Dreccer *et al.* (2000b) showed that oilseed rape had higher efficiency to produce the biomass for N uptake but have lower harvest index than canola. Sub-optimal as well as supra-optimal N nutrition has a negative effect on the final yield of canola (Barlóg and Grzebisz 2004). There is still considerable uncertainty about N management in canola. Some researchers have argued that seed yield does not simply depend upon the dose of applied N but also on the timing of N application (Hocking *et al.* 1997c; Sieling *et al.* 1998a). Under the rainfed condition of Australian agriculture, a fertiliser rate of 40-60 kg N ha^{-1} is widely used with some adjustment based on N fertility of the soil. It is still unclear what impact targeting a specific phenological growth stage for N application will have on water use, N use efficiency and productivity in terms of dry matter and seed yield under water limited conditions. Therefore the aim of this study was to improve our understanding of physiological limitations to the yield of canola in rainfed cropping system in relation to water use efficiency.

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Chapter 2

2. Review of Literature

Plant breeding and improved agronomic management have contributed to yield improvement of canola and mustard in Australia. Nitrogen is an expensive and difficult to manage input in rainfed farming systems with variable amounts and distribution of rainfall, but it is critical to achieving high yields in canola and improving crop water use. Application of N at critical phenological growth stages may be the key to success for managing the available water, resulting in increased seed yield through improving the nitrogen use efficiency (NUE) and water use efficiency (WUE).

In this thesis it is proposed that applying N at critical phenological growth stages to match the N demand of the crop, combined with better management of available water, can improve the seed yield and efficiency of N and water use. The ultimate goal of this study, as for most of rainfed farming systems, is to optimise the balance between available water and N. This depends largely upon crop canopy development and management. The purpose of this review of literature is to identify the knowledge gaps responsible for low and variable seed yield of canola and their relationship with low NUE and WUE in Australian conditions.

2.1. Canola cultivation in Australia

Canola is the main broadleaf rotation crop in the cereal producing regions of Australia. Canola production in canola grew from 100,000 ha in 1990 to 1.4 M ha in 1998 (Colton & potter 1999). In 2013, canola was grown on an area of 3.3 M ha in Australia. Average area under canola has remained around 1.6 M ha for the period of 1998-2013 and has never dropped below 1 M ha since 1990. Canola is an established crop in the areas of southern Australia with growing season rainfall of 400mm and above. Typically canola has been grown in areas with growing season rainfall higher than 450 mm but Carmody and Cox 2001 showed that it is possible to grow canola in areas with low rainfall (approximately 325 mm). Adoption of early maturing cultivars has pushed canola production into low rainfall areas of the Australian grain belt. However profitability depends upon a number of factors; soil constraints, onset of rainfall, weed competition, disease, high temperature at flowering and pod filling and management of nutrient, weed, insect pest and harvest. Effective management of these yield limiting factors is the key to profitable canola production in low rainfall areas of Australia (Carmody and Cox 2001).

2.2. Morphology, Growth and Development of canola

Canola is an indeterminate crop plant. Colton and Sykes (1992) divided life cycle of canola into seven main stages (0-6) (Fig 1) where as BBCH growth scale describes ten main growth stages (0-9) (Lancashire *et al.* 1991).

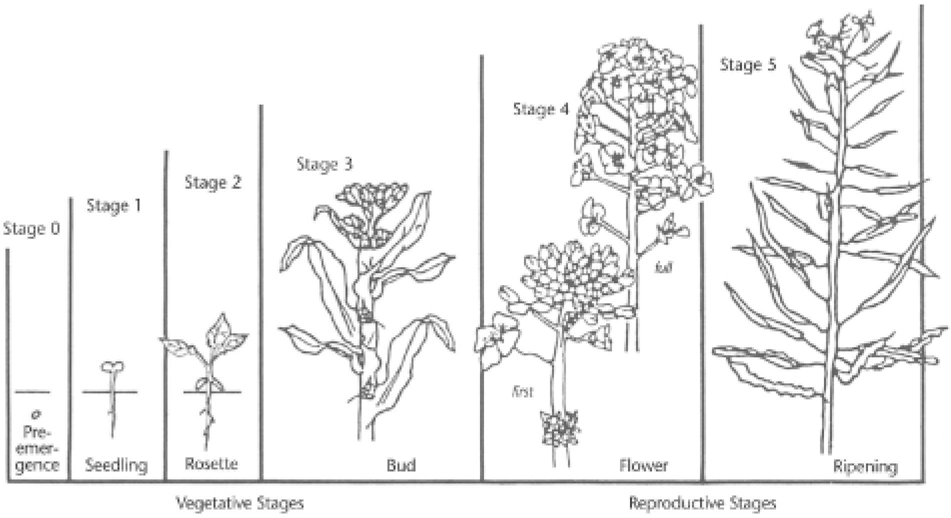

Figure 1 : Growth Stages in B. *napus* and B. *juncea.* Source: CCC (2003)

In brief, initial stage is the germination and emergence where seed absorb moisture and root split the seed coat and shoot emerges. After germination a thin tap root supports the plant and plant starts to produce leaves. A fully grown canola plant can have 10-15 leaves on main stem (Colton and Sykes 1992). Each node of stem has a single leaf with a spacing of 5-10 cm. Height of earlier canola varieties were about 1.2-1.5 m whereas modern TT verities are much shorter at 0.7-1.0 m long (e.g. Fighter TT) and some hybrids and conventional verities are 1.5-1.7 m tall (e.g. AV Garnet, Hyola575cl). After stem elongation, flower bud development occurs. Flower buds remain enclosed in leaves from the early stem elongation to green bud stage. As stem elongation progresses flower bud emerges and flower become free from leaves. Flowering starts on terminal racemes and ends when buds become visible on the side branches. Canola flowers develop on terminal racemes and are bisexual with 4 sepals and 4 petals. Canola seed develops in 2-celled pod (or silique) with a prominent mid–vein (Bailey *et al.* 1976). Entomophilous flowers of canola are capable of both self and cross pollination (Treu *et al.* 2000). High pollen production within the flower generally outcompete the cross pollination but still outcrossing up to 30% can occur between adjacent plants. The fruiting bodies of canola are siliques, commonly called pods (Mendham *et al.* 1984). A single pods consists 10-15 canola seeds. Seed development starts from the lower branches. Seeds remain translucent until they reach their final size and become green and finally become hard and turn black/brownish (Colton and Sykes 1992). This stage when seed colour changes is recognised as the physiological maturity and crop harvesting occurs.

2.3. Yield trends in canola and mustard in Australia

The average seed yield of canola has doubled globally and in Australia in the last four decades, with an average increase of 27 kg ha^{-1} yr $^{-1}$(Rondanini *et al.* 2012). However, long term (1972-2013) yield data from Australia shows high variability in canola yield (FAO 2014). Rondanini *et al.* (2012) found that Australia has the highest yield variability among the 12 main canola growing countries with relative yield residuals ranging from 0 to 60%. However a more detailed examination of the long-term trends suggests a slowing in yield gains in recent times (Fig 2). The national yield in the period 1972-2013 has averaged 1.1 t ha^{-1} (range 0.3-1.7 t ha^{-1}). Dividing this period into two phases (a) conventional canola growing era 1972-1989 and (b) the era of improved hybrid canola cultivars with different weed control options, shows two distinctive patterns of yield improvement. Prior to 1989, the average rate of yield improvement was 55 kg^{-1}ha^{-1}yr^{-1} while in the second phase there was no improvement in seed yield and there was some evidence of a decline in canola yield (Fig:2). This national trend is evident in each State although the magnitude of the effect varies. State wise average seed yield of canola is 1.3 t ha^{-1} for South Australia and 1.2 t ha^{-1} for New South Wales and Victoria 1.0 t ha^{-1} for Western Australia, 1.1 t ha^{-1} for Tasmania and 0.5 t ha^{-1} for Queensland.

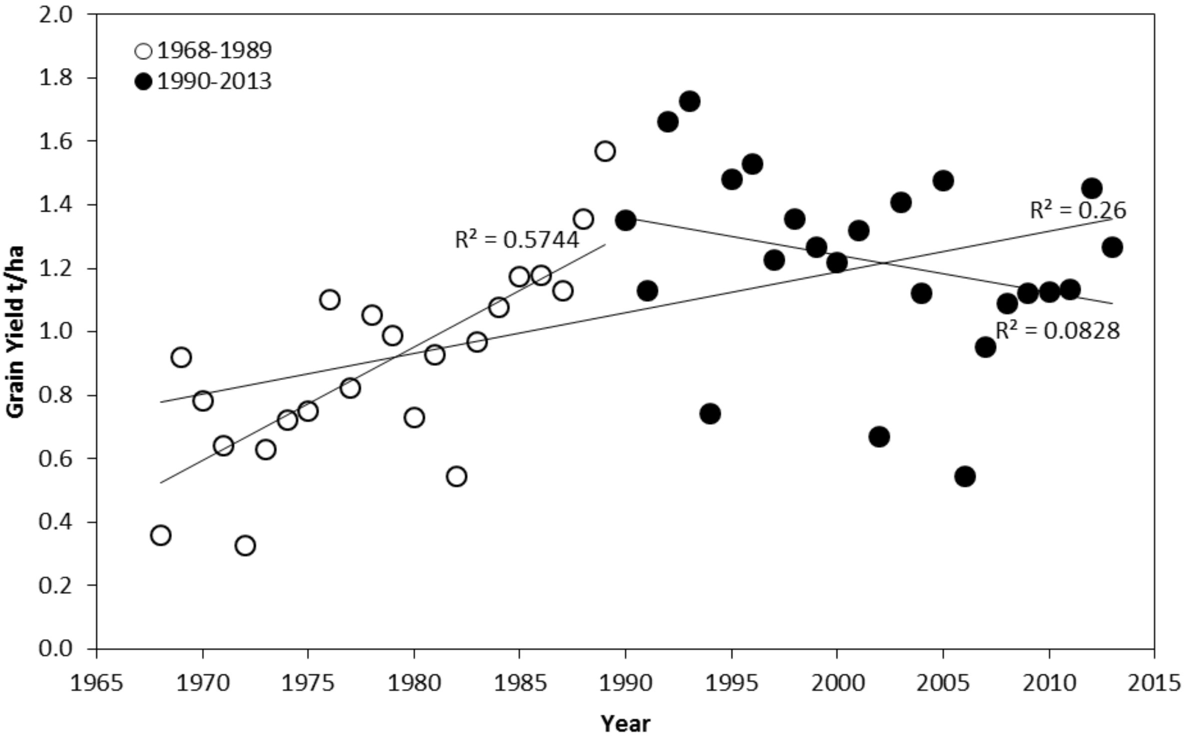

Figure 2: Average Australian yield data for canola from 1972-2013. The open symbols represent the average annual yield prior to 1989, where closed symbols represent the annual average yield from 1990-2013. Linear lines show the improvement or decline in annual yield over different time spans. (FAOSTAT, 2014).

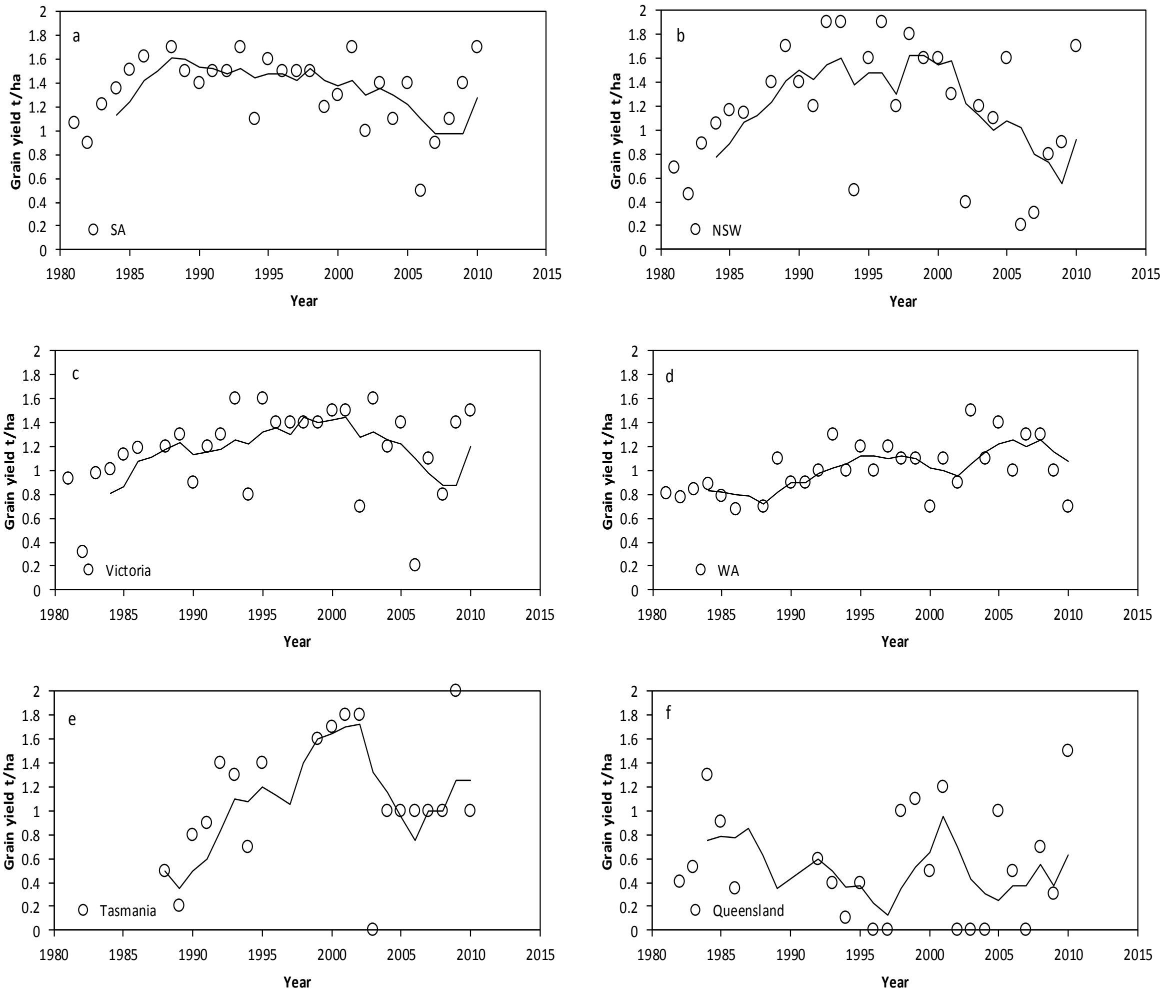

Figure 3: State- wise seed yield trends of canola in Australia from 1981-2010 (a) South-Australia (b) New South Wales (c) Victoria (d) Western Australia (e) Tasmania (f) Queensland (FAO 2014)

In low rainfall areas many of the constraints to yield are directly and/or indirectly related to the water use pattern and uptake of available N. Although most of the main canola growing states are close to the national average, the year to year variability in canola yield is high. Canola yield for South Australia ranges from 0.5 t ha^{-1}to 1.7 t ha^{-1}(Fig: 3a), New South Wales from 0.2 t ha^{-1}to 1.9 t ha^{-1}(Fig: 3b), Victoria from 0.2 t ha^{-1}to 1.6 t ha^{-1}(Fig: 3c), Western Australia from 0.6 t ha^{-1}to 1.5 t ha (Fig: 3d), Tasmania from 0 t ha^{-1}to 2 t ha^{-1} (Fig: 3e), and Queensland from 0 t ha^{-1}to 1.5 t ha^{-1}(Fig: 3f). It is also evident that the average yields of canola have not improved despite the introduction of hybrid varieties with greater potential yields and regular use of N fertiliser. Data from the last 24 years show much greater variation in yield of canola as compared to the area under cultivation. Even in the medium and high rainfall areas, there is concern about yield variation and in some cases declining yield has been noticed. According to Kirkegaard *et al.* (2006), yield decline in high rainfall years was mainly due to the disease, blackleg (*Leptosphaeria maculans*) and sclerotinia stem root (*Sclerotinia sclerotiorum).* In addition, other possible causes for yield decline may be increased crop intensity with reduced tillage, which may increase the soil compactness and hence lower rooting depth (Mead *et al.* 2005). Mead *et al.* (2005) found that most of the crops yield was not limited due to N availability but was greatly influenced by the seasonal water availability. However it can be argued that limited water supply can hinder the N response, so crop still can be N stressed under limited water conditions. Despite being the major broadleaf crop in Australia, canola struggles with low yield and high yield variation. This indicates that in the rainfed cropping system of Australia, the yield potential of canola is not being met. There are several constraints related to environmental stress that could be responsible for the gap between attained yield and the yield potential of canola. In low rainfall areas many of these constraints are directly and/or indirectly related to the water use pattern and uptake of available N in canola.

2.4. Phenology and adaptation

In Australia, canola is sown in autumn predominantly with spring type varieties and the crop matures within 5-7 months with a limited amount of water in most areas (Walton *et al.* 1999). Varieties differ in their time to flowering and their response to sowing date, both of which influence the environmental conditions under which the crop grows. The timing of water deficits in relation to the key growth stages is important in determining the yield of a crop and its WUE. Crop phenology is a basic tool to study the impact of environment on the development of the crop. Phenology of canola varies with genotypes, broadly classified as spring and winter cultivars (Burton *et al.* 2008). Rates of development can also be altered in the field by adjusting the time of sowing. Early sowing helps in early flowering to avoid water stress and by providing more time during the stem elongation phase which could increase crop yield (Thurling and Kaveeta 1992). In rainfed conditions in Australia, where early flowering and a longer period of inflorescence development can help to achieve better yield and to minimise the risk of crop failure (Walton *et al.* 1999), flowering and inflorescence appearance are the key parameters in canola phenology.

The major controls of flowering have been described by Mendham and Salisbury (1995). There is an initial vegetative phase in which a minimum number of leaves need to be initiated before floral initiation occurs and subsequent development depends on the vernalisation and photoperiod response of the crop. There is significant genetic variation in responsiveness to both vernalisation and photoperiod in canola and mustard. The most appropriate combinations of vernalisation and photoperiod sensitivity in genotypes reflect the environment in which the crop grows and the times of sowing most commonly used. Salisbury and Green (1991) showed that European cultivars were most responsive to vernalisation whereas the Australian cultivars were intermediate and Canadian cultivars were least responsive. In typical Australian conditions, average photoperiod from emergence to flowering varies between 11.0 h to 13.0 h. Robertson *et al.* (2002a) analysed the photoperiod response of 21 Australian canola cultivars and found the response occurred between 10.8 and 16.3 h, and plants responded to photoperiod from emergence without having a pre-inductive phase. In addition, vernalisation can show an additive effect with photoperiod (Mendham and Salisbury 1995). Spring cultivars grown in Australia do not need vernalisation to flower but nevertheless they are responsive to it (Walton *et al.* 1999). Hence the role of vernalisation and time of sowing with different cultivars can be an effective tool for breeders to select the desired trait as well as for the physiologists and agronomists studying canola adaptation. The pattern of crop development influences the amount and pattern of dry matter production and hence demands for N and the pattern of crop water use.

2.5. Yield determination and effect of water and N on different growth stages of canola

Yield formation in indeterminate plants is a complex process which develops through the season, so each growth stage contributes to the final seed yield. Based upon the magnitude of their contribution to yield, some stages have been identified as critical growth stages. For example, Dreccer *et al.* (2000a) found flowering and pod development to be the most limiting growth stages in yield formation. Development of canola is divided into two main phases: vegetative and reproductive based on the stage of development of the shoot apex, although the two phases are not completely independent. Growth during the vegetative phase influences growth and yield potential that develops during the reproductive phase. Nitrogen uptake and utilisation are important in both phases for various structural, metabolic and reproductive processes (Hirel *et al.* 2007). The availability of N and water can affect these important processes. The role of N and water and their effects on several growth stages and yield formation will be discussed in following sections.

2.5.1. Germination

Germination and emergence determine plant density which can have a major impact on yield and its stability (Sierts and Geister 1987). Larsen *et al.* (1998) reported that time to germination was significantly correlated with seed yield. Other studies have shown that the time to germination tends to be influenced by genetic (Acharya *et al.* 1983; King *et* *al.* 1986) and environmental factors (Kondra *et al.* 1983). Leterma (1988) (cited in Gabrielle et al 1998) estimated that 130-140 ˚Cd is required for the emergence of oilseed brassica using 0˚C as the base temperature. The requirements for N during germination are met by the use of the N reserves in the seed rather than an external supply of N. However, high rates of N fertiliser can reduce germination and establishment of canola by its influence on water uptake through osmotic effects and due to the toxic effect of ammonia. Therefore a low N rate of 10 kg ha^{-1} as a basal dressing is suggested in medium and low rainfall areas, to mitigate the risk of germination failure (Potter *et al.* 2009).

2.5.2. Early leaf development

In canola and mustard the first true leaf appears 4-8 days after emergence, after which plants quickly progress to the rosette stage with older leaves at the base and new leaves in the centre (CCC 2003). Genetic difference in phenology and early vigour can play a role in N uptake and utilisation and hence yield potential. Winter cultivars produce around 12 leaves (Smith and Scarisbrick 1990) whereas spring cultivars produce only 7 leaves (Morrison and McVetty 1991) prior to floral initiation. At this stage, young leaves and roots develop quickly and act as the main sink organs for synthesis and assimilation of amino acids due to rapid N uptake, (Hirel and Lea 2001). This leads to the highest N percentage in dry matter at the rosette stage (Parker 2009).

During the juvenile growth stage (rosette + stem elongation), the supply of N increases the leaf area index (LAI) and crop growth rate (CGR) which ultimately results in a higher number of pods per plant (Allen and Morgan 1972). However, the effect of these changes was minor on later formed yield components like seeds pod^{-1} and seed weight (Hocking and Stapper 2001). Yau and Thurling (1987) also found an improvement in leaf area and net assimilates with the application of N. The availability of N during the early growth and development has an important role in establishing and maintaining a photosynthetically active leaf canopy throughout the growing season (Rathke *et al.* 2006), which becomes a major influential factor during the reproductive stage (Rossato *et al.* 2001). However, higher N supply does not greatly affect the photosynthetic capacity per cm^2 leaf area of the crop but rather improves the crop productivity by increasing and maintaining LAI and extending the photosynthetic period (Rathke *et al.* 2006). A higher LAI leads to higher dry matter accumulation which later supports the pod growth and final yield (Brar and Thies 1977). It is assumed that at the leaf development stage canola uses most of the N to increase the leaf area and leaf thickness to harvest more photosynthetically active radiation (CCC 2003). Several studies have reported that increased N supply and good soil fertility increased the weight and chlorophyll content of the leaves of oilseed rape (Ogunlela *et al.* 1989; Gammelvind *et al.* 1996). Studies based on the plant N status at different growth stages showed the strong relationship between N content of canola during the early developmental stages (rosette to green-bud) and seed yield (Hocking 1987; Bernardi and Banks 1993).

2.5.3. Dry matter formation (side shoots, stem elongation, inflorescence and flowering)

Stem elongation in canola generally starts when the ninth leaf unfolds and during this phase the canola plant reaches its maximum stem length. Sometimes early growth stages (rosette and stem elongation) overlap and there may be some overlap with early flowering (CCC 2003). During this period stem and leaf growth, flowering and pod initiation occur simultaneously and to meet the energy requirement of these competing processes the plant needs an increasing supply of water and N. This results in greater vegetative growth and is a probable reason for the low harvest index commonly found in canola and mustard. In canola and mustard, maximum leaf area is reached when flowering begins but subsequently it starts to decline due to shading of the lower leaves. At this stage the lower leaves become a source of nutrition for the rest of the plant (CCC 2003) as uptake of N may not be sufficient to meet the demands of the crop as biomass continues to increase. When flowering begins the lower leaves and the lower section of the main stem have a higher concentration of N (Kullmann and Geisler 1986). Recently Rossalo et al. (2001, 2002) found that a 23 kDa protein acts as a vegetative storage of N in the tap root up to the flowering stage, which is later hydrolysed when seed filling begins, as cited in (Rathke *et al.* 2006). Later, this pool of N supports the pod set and a few more secondary branches at later stages of crop growth with N re-translocation from leaf to flower. A decline in the nutritional supply to the plant at this stage may be a cause of higher leaf senescence which affects the final seed yield. (Diepenbrock 2000) describes the falling of leaves at flowering as the “most limiting process” for seed yield in the development of canola.

2.5.4. Flowering

Flowering is the most critical stage for the oilseed brassica. Any stress at this stage is likely to lead to lower yield. During flowering, branches continue to grow and flowers develop into pods. Loss of N accumulated during the early vegetative phase occurs due to shedding of the leaves (Malagoli *et al.* 2005). Canola plants often initiate more buds than they can develop into productive pods (Tayo and Morgan 1975). Abortion of flowers and pods is very common and is a natural feature at this stage, but it is sensitive to environmental conditions and increases under low N stress and dry conditions. It has also been seen that early flowering leads to more productive pods especially on the main stem and first three secondary branches (CCC 2003). Furthermore, flowering is a critical period for management as it is the final stage of growth before pod setting. At this stage a healthy crop creates opportunities for better seed and oil yield and N supply is a key factor in maintaining plant health at this stage.

All the above growth stages which lead up to pod set and source for seed fill can be considered as the stages that lead to the development of the sink in the canola plant, and hence its potential yield: a greater sink increases the chances of higher yield in canola. Nitrogen supply and uptake has an important role in developing the sink of the crop by affecting growth at different stages of development.

2.5.5. Development of fruit, ripening and senescence

According to Habekotté (1997), plant characteristics responsible for better absorption of light and synchronisation between source and sink are important for high canola yields. At the late flowering stage in oilseed brassica, competition for resources begins between the flowers and pods (CCC 2003). The study of Diepenbrock and Grosse (1995) showed pod development and seed set occurs largely after completion of N absorption by the plant. Utilization of water at this stage increases and reaches its peak (CCC 2003). Rossato *et al.* (2001) showed that endogenous N is redistributed to stem and roots and acts as the buffering storage for the use of reproductive organs at later stages. Rathke *et al.* (2006) also found that during pod development, the source of assimilate supply shifts from leaf to the stem with little contribution from the newly developing pods. Hocking and Stapper (2001), found that at this stage pod walls can act as a resource for N by supplying up to 25% of the N requirement of the seed. Any stress at this stage can lead to reduced yields caused by aborting pods or reduced number of seeds per pod. At flowering, potential pods and seeds per pod have been determined but the final number remains uncertain until the later stage of ripening, as canola plants need an uninterrupted supply of water and nutrients right up to senescence to achieve the maximum seed yield (CCC 2003). In brief, the significant role of N after anthesis in canola to maintain photosynthesis has been recognised (Rathke *et al.* 2006), but its specific effects on seed yield and its interaction with post-flowering environmental conditions remain unclear.

2.6. Cultivation practices in Australia

In Australia only spring type canola verities are grown. Rainfed crops are sown with the onset of rain in April and May called season break. Growing season of canola is about 5-7 months and most of the Australian varieties flower in 6-8 week time after sowing and ripen in late spring (Walton *et al.* 1999). Growing season of canola in Australia is shorter than Europe but longer than Canada. In Europe growing season of canola is about 12 months due to vernalisation requirement of European cultivars, whereas in Canada growing season is short (approximately 4 months) due to longer day length and warm conditions (Walton *et al.* 1999). The average seed rate of canola is 4-6 kg ha^{-1} with the exception for hybrids, which are sown at around 3 kg ha^{-1}. These seeds rates are used to achieve a plant population of 50-70 plants m^{-2}. Low seed rates are also used for varieties with small seed and in low rainfall areas where low plant population (35 plants m^{-2}) is recommended. Normal sowing depth is 2 to 4 cm under optimal moisture conditions for rapid emergence, deep sowing up to 6cm is also used in situations where surface soil is dry and soil temperature is high. Australian varieties are reasonably frost tolerant. Early frost can damage the seedlings whereas unusual late frost during post-flowering period can damage and abort seeds and reduce yield (Walton *et al.* 1999).

Canola needs 30% more nitrogen than cereals. In Australian conditions canola needs 40-50 kg N, 8 kg Phosphorus and 10 kg sulphur per ton of seed produced (Norton 2003). However the analysis of imidazolinone and triazine canola data from national variety trail sites in medium and high rainfall across Australia showed that most of the time 50-100 kg N ha^{-1}was applied to canola crop irrespective of initial soil N status (Table:1a).

In Australia some N is usually applied at sowing and the rest is top-dressed onto the crop during growing season. Most of the farmers consider splitting as the financial risk management tool rather than for improving the efficiency of N use. The amount of fertiliser application at sowing through the seed drill depends upon factors like row spacing, temperature and soil moisture.

Table 1a: Canola yield, N management and N use efficiency for applied fertiliser, soil N at sowing, productivity and growing season rainfall data from high rainfall sites SA (Bordertown), NSW (Wagga wagga, Temora), and Victoria (Diggora). Sites were classified as high rainfall fall where annual rainfall is higher than 450mm. (National Variety Trials 2015).

	Mean Yield (t ha^{-1})	Applied Fertiliser N (kg ha^{-1})	NUE$_{SY\ F}$$^{-1}$ (kg kg^{-1})	Splits	Soil N 0-10 cm (mg kg^{-1})	Soil N 10-60 cm (mg kg^{-1})	Productivity (Kg kgN^{-1} mm^{-1})	GS Rainfall (mm)
Imidazolinone								
NSW	2.0	83.5	26.7	2.7	45.4	21.5	0.1	270.2
SA	1.9	84.8	23.2	3.0	50.8	11.0	0.1	360.2
Vic	2.5	66.6	48.3	2.8	55.2	21.5	0.2	278.9
WA								
Average	2.1	78.9	32.0	2.9	50.1	19.7	0.1	293.6
Triazine								
NSW	1.7	90.0	23.0	2.9	42.9	22.0	0.1	268.4
SA	1.7	84.8	20.3	3.0	37.6	9.3	0.1	360.2
Vic	2.4	66.2	71.7	2.5	52.9	22.5	0.3	278.4
WA								
Average	1.9	81.1	36.7	2.8	44.8	19.2	0.2	295.1

Data from NVT trials showed that two splits (at sowing and topdressing later) are common in medium rainfall areas whereas three splits are common in areas with high rainfall. Yield of canola is almost always limited by the water availability during seed filling and ripening (Walton *et al.* 1999). So the maturity and final yield is dependent on water availability during the post-flowering period. In early 2000s, swathing or windrowing was the universal option for canola harvest (Carmody and Cox 2001) but quite recently direct harvesting is being tried by many growers.

Table 1b: Canola yield, N management and N use efficiency for applied fertiliser, soil N at sowing, productivity and growing season rainfall data from medium rainfall sites of SA (Turretfield and Yeelanna), Victoria (Minyip) and westeran Australia (Williams). Sites are classified as medium rainfall fall where annual rainfall is less than 450mm . (National Variety Trials 2015).

	Mean Yield ($t\ ha^{-1}$)	Applied Fertiliser N ($kg\ ha^{-1}$)	$NUE_{SY\ F}^{-1}$ ($kg\ kg^{-1}$)	Splits	Soil N 0-10 cm ($mg\ kg^{-1}$)	Soil N 10-60 cm ($mg\ kg^{-1}$)	Productivity ($Kg\ kgN^{-1}\ mm^{-1}$)	GS Rainfall (mm)
Imidazolinone								
NSW								
SA	2.3	77.8	32.6	2.4	40.3	6.8	0.1	319.5
Vic	1.7	36.8	59.4	1.8	30.2	12.1	0.3	206.3
WA	2.5	71.1	40.9	3.5	69.0	33.3	0.1	361.1
Average	2.2	66.2	41.0	2.5	45.6	16.6	0.2	299.5
Triazine								
NSW								
SA	2.0	77.1	27.7	2.4	39.0	8.5	0.1	306.4
Vic	1.6	43.0	51.2	2.0	30.2	17.7	0.3	206.3
WA	2.1	66.7	34.6	3.0	58.0	25.2	0.1	345.3
Average	2.0	68.5	33.6	2.5	44.2	18.3	0.1	295.3

2.7. Nitrogen use efficiency

Nitrogen use efficiency received considerable attention from researchers in the late 1970s when Grami and LaCroix (1977) reported N uptake is an inherited trait in oilseed Brassica. Efficiency of N utilisation in farming systems is crucial for yield, quality, economics of production and reducing environmental impact of N fertiliser use (Campbell *et al.* 1995).

Conventionally, NUE is considered in terms of the efficiency of uptake and the efficiency of utilisation. Several different parameters have been used in the past to identify efficient use of N (McDonald 1989; Fageria and Baligar 2005; Rathke *et al.* 2006):

$$\text{N use efficiency for seed yield } (\text{NUE}_{\text{SY}})\ (\text{kg kg}^{-1}) = \frac{\text{Seed yield}}{\text{N supply (Fertiliser + Soil N)}}$$

$$\text{N uptake efficiency } (\text{kg kg}^{-1}) = \frac{\text{Total N uptake}}{\text{N supply}}$$

$$\text{Agronomic efficiency (kg kg}^{-1}\text{)} = \frac{\text{SF} - \text{Sc}}{\text{F}}$$

$$\text{Apparent recovery (\%)} = \frac{\text{NF} - \text{Nc}}{\text{F}} \times 100$$

$$\text{Physiological efficiency (kg kg}^{-1}\text{)} = \frac{\text{YF} - \text{Yc}}{\text{NF} - \text{Nc}}$$

Where SF and SC are the seed yield of the fertilised (F) and unfertilised (C) plots, YF and YC are the biological yield of the fertilised and unfertilised plots, NF and NC is the N contained in biological yield (kg ha^{-1}) of fertilised and unfertilised plots, and F was the amount of fertiliser N applied as granular urea.

In Australia, Yau and Thurling (1987) analysed 40 spring rapeseed genotypes and reported significant differences in N utilization. Svečnjak and Rengel (2006) and Balint *et al.* (2008) also reported that the extent of response to N can vary among cultivars. However, there is still a scarcity of data for genetic variability in NUE at low N fertilisation (Hirel *et al.* 2007). It is well known that some plant species and genotypes can be more nutrient efficient on nutrient deficient soils than other species and/or cultivars (Gerath and Schweiger 1991; Rengel 1999; Gan *et al.* 2008). It is assumed that in nutrient efficient plants some specific physiological mechanisms are used to access sufficient amount of nutrients from nutrient deficient soils or by improved utilisation efficiency (Sylvester-Bradley and Kindred 2009). Even though several studies indicated that nutrient use efficiency has been improved in canola by breeding and agronomic management (Yau and Thurling 1987; Svečnjak and Rengel 2006; Balint *et al.* 2008; Gan *et al.* 2008), Fageria *et al.* (2008) argued that the performance of current cultivars for the efficient use of nutrients is far from ideal. Sylvester-Bradley and Kindred (2009) compared some major crops for their NUE and found that oilseed rape was one of the low N efficient crops with NUE = 9 kg DM kg $N^{-1}_{\text{available}}$ compared to sugar beet (NUE = 69 kg DM kg $N^{-1}_{\text{available}}$) or potato (NUE = 40 kg DM kg $N^{-1}_{\text{available}}$). Norton and

Wachsmann (2006) found that NUE of applied fertilisers is reduced with higher N rate. In their three experiments $\mathrm{NUE}_{\mathrm{SY}\ \mathrm{F}}{}^{-1}$ varied between 5.7–12.1 kg kg^{-1} N. Moreover the NVT trial data also showed similar values for $\mathrm{NUE}_{\mathrm{SY}\ \mathrm{F}}{}^{-1}$. The response of canola to applied fertiliser in Australian conditions is close to that reported in UK by Sylvester-Bradley and Kindred (2009)

It has been generally seen that nutrient efficient cultivars were more responsive to applied fertiliser N at low rates (Yau and Thurling 1987), which may be related to differences between the cultivars in N uptake and translocation (Grami and LaCroix 1977). Nitrogen harvest index (NHI), a ratio between the translocation of N from dry matter to seed, is often poor in most of the crops and generally remains around 30-40% (Raun and Johnson 1999; Hirel *et al.* 2007), although a wide range of NHI has been reported for canola varying from 10% (Malagoli *et al.* 2005; Sylvester-Bradley and Kindred 2009) to 85% for canola and 90% for Indian mustard (Hocking and Stapper 2001). These latter values are exceptionally high and much higher than other reports. Studies have shown that improvement in yield through plant breeding has been limited by the inverse genetic relationship between protein and oil content for canola seed (Brennan *et al.* 2000; Jackson 2000). Nevertheless Gerath and Schweiger (1991) have found some cultivars which have higher N uptake and higher yield without any negative effect on oil concentration. The mechanisms for improvement in NUE in canola are not well understood; a crop with maximum NUE would be expected with maximum photosynthetic activity with minimal/no fertiliser input and given amount of available water. This is a massive challenge to overcome as the present performance of canola is far from ideal (Sylvester-Bradley and Kindred 2009). It is also clear that for improvement in NUE and seed yield without compromising the oil concentration of canola, improved agronomic management strategies for N are required (Rathke *et al.* 2006).

The main components of N management in the field are placement, rate and timing of application (Malhi *et al.* 2001). Research shows that there is little difference in the utilization and efficiency with respect to the form in which the N is applied (Johnston *et al.* 1997; Grant *et al.* 2002a). Hocking *et al.* (1997b) found the uptake of N in canola was 1% at the rosette stage, 32% at the bud visible stage and 56% at the start of flowering. The amount of N application affects the total N uptake as well as the yield of canola and placement of fertiliser is beneficial for reducing N rate (Malhi and Nyborg 1991; Grant *et al.* 2002a), but for dryland canola production seasonal rainfall amount and pattern could also affect NUE. Higher N rates have been related to low N utilisation efficiency, especially when N rate increased beyond 100 kg N ha^{-1} (Gan *et al.* 2008). Crop demand for N increases greatly after the bud visible stage so synchronisation of N application with crop demand has the potential to improve nitrogen use efficiency (Fageria and Baligar 2005; Rathke *et al.* 2006; Mahli *et al.* 2007). All genetic, agronomic and environmental factors (temperature, radiation and soil moisture and nutrient level) should be taken into consideration for improved NUE of canola.

2.8. Water use efficiency

Plant growth depends upon the water supply due to the common pathway for transpiration and carbon assimilation (Kramer 1969), which leads to unavoidable water loss through stomata (transpiration) as atmospheric CO_2 moves into the leaf. There are a number of ways of looking at water use efficiency (WUE). At a leaf or plant level, WUE can be calculated as the ratio between the total amount of CO_2 fixed per unit of water transpired and evaporated from soil surface. However, for an agronomist, it can be calculated as seed yield produced with the total water supply over the growing season, while for a farmer, it can be seed yield per mm of rainfall (Perry *et al.* 1991). Evaluation of WUE and comparing it with benchmark values can play a significant role in the identification of potential constraints to yield other than moisture stress (Cocks *et al.* 2001). Apart from its direct effect on biomass production and yield, water supply also interacts with N supply: greater water availability can increase N uptake by the plant and enhances NUE (Kappen *et al.* 2000).

Generally mustard has been found to use less water than canola under drought conditions (CCC 2003).Therefore, mustard could be useful oilseed crop for drier rainfed environments but this needs field evaluation in South Australia. Norton and Wachsmann (2006) found a strong relationship between dry matter and water transpired with different N levels but a poor relationship between seed yield and water use. They argued that seed yield of canola can be improved by reducing soil evaporation, which will provide more water for transpiration. They further suggested that transpiration of the crop can be improved with agronomic management by increasing the crop density or improving the early vigour of the crop to cover the bare ground. However, increased crop water use during early growth stages may lead to water scarcity in later developmental stages which in turn may become a yield limiting factor in medium and low rainfall areas. Hence correct balance between pre- and post-flowering water use of canola needs to be determined.

There have been few attempts to understand the relationship between canola yield and seasonal water supply and their effects on WUE in the Australian environment. Hocking *et al.* (1997e) found WUE of 13 kg $ha^{-1}mm^{-1}$ for canola which was later benchmarked to 15 kg $ha^{-1}mm^{-1}$ by simulation modelling (Robertson and Kirkegaard 2006). However, Cocks *et al.* (2001) showed that WUE of canola varied from 3-18 kg $ha^{-1}mm^{-1}$ over 3 seasons in their experiments in northern Australia and concluded that variation in WUE was due to the timing and amount of rainfall.

Apart from the above studies, no noticeable effort has been made to understand WUE in canola. However, WUE has been studied extensively in wheat (Cornish and Murray 1989; Angus and Van Herwaarden 2001; Rodriguez and Sadras 2007; Hochman *et al.* 2009; Sadras and McDonald 2012; Sadras *et al.* 2012), with the widely adapted approach to calculate the potential yield from available water by French and Schultz (1984). However the French and Schultz technique has the know limitations e.g. this approach was originally developed using the cultivars and management practices of 1960s and 1970s and estimated a slop of 20 kg $ha^{-1}mm^{-1}$ whereas the current cultivars are close to 25 kg $ha^{-1}mm^{-1}$ and management practices have changed considerable. Moreover French and Schultz explained the soil evaporation as 60% of seasonal rainfall (commonly cited as 110 mm for South Australian environment) whereas Sadras and McDonald (2012) explained that it can vary significantly between 35mm to 200 mm with the location and seasonal conditions. In South Australia, where the problems of runoff and deep drainage are negligible, this new approach defined the potential yield based on a boundary functions fitted to empirical data. This provided a framework in which to explore water–limited yield in wheat in terms of a threshold level of water use before yield was accumulated (which approximated soil evaporative loss) and potential transpiration efficiency (TE) of 22 kg $ha^{-1}mm^{-1}$ once this threshold was reached. The same framework was applied to canola by Robertson and Kirkegaard (2006) with help of modelling tools to define the water-limited potential yield, with a threshold of 120 mm and a TE of 15 kg $ha^{-1}mm^{-1}$ (Robertson and Kirkegaard 2006). This approach may explain the water limited potential yield of canola and its relationship with seasonal rainfall by taking into account initial soil moisture.

2.9. N-water co-limitation theory

Nitrogen and water are main factors limiting crop yield under Mediterranean environments (Cossani *et al.* 2010). It will be difficult to realise the full potential of genetic improvement without improving the N uptake and water use of these crops (Sinclair and Rufty 2012). Water availability has a large influence on crop demand for and response to N. The availability of N depends on pre-sowing mineralization of N and within season mineralization of organic N, whereas water is always a limiting resource and its availability depends on the total rainfall during the fallow and growing season, which vary seasonally. Effective water use is vital to mitigate the gap between actual and maximum attainable WUE under rain-fed systems (Sadras and Angus 2006) and hence will reduce the yield gaps (defined as the difference between actual and attainable seed yield). Attainable yield is the yield of crop which can be achieved by using all improved technology with skillful management of the available resources. On the other hand N yield is a function of plant available water (Campbell *et al.* 2004). So water deficits can limit N response in plants by reducing the N uptake and utilisation (Benjamin *et al.* 1997). Many studies have shown these effects of water availability on N response of crops and *vice-versa* (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012). The absorption, translocation (Malhi *et al.* 2007) and partitioning (Jackson 2000) of N improves with better utilisation of available water, i.e. WUE. Sadras (2004) found that the gap between actual and potential yield was lower when water and N equally co-limited the growth of wheat crop. Bloom *et al.* (1985) proposed that plants control the allocation of resources so that growth is equally limited for all resources. Consequently, growth of the plant will be maximised when all resources are equally limiting. In other words, degree of co-limitation of the resources would positively relate to the growth of given population. The presence of co-limitation has been identified in systems from cell to biomes (Venterink *et al.* 2001; Flynn 2002; Maberly *et al.* 2002). Several studies identified that co-limitation in a system arises and is influenced by several mechanisms, including the interaction between the different components and factors of the system. It has been also identified that the degree of co-limitation changes with time with the interactions between different factors and components (Sinclair and Park 1993; Berman and DeJong 1997; Maberly *et al.* 2002; Sadras 2004). Based upon the economic analogue of Bloom *et al.* (1985) for resource limitations, the hypothesis of negative relationship to the degree of water and N co-limitation (C_{WN}) with the yield gap (defined as the difference between actual and attainable seed yield) has been supported by empirical and simulation studies in wheat and barley(Sadras 2004; Cossani *et al.* 2010).

The work so far on water and N co-limitation has been conducted in wheat and barley with a fixed amount of N applied pre-sowing whereas in rainfed environments post-sowing N management is an important tool for risk management and for improved NUE and WUE in canola and mustard.

2.10. Site specific agronomic management to improve the seed yield

As argued in earlier sections, yield of canola is almost always limited by the water availability in medium and low rainfall areas. So the effective management of water can improve the seed yield of canola. Rate and timing of N can be used as the tool to manage water partitioning hence it can improve the seed yield, NUE and WUE. This section will discuss some site specific agronomic management to improve the seed yield and its effects on NUE and WUE.

2.10.1. Time of sowing

Time of sowing is an important determinant of yield. In Mediterranean environments early sowing helps to avoid high temperature and drought stress at the end of season that curtails growth whereas late sowing reduces the risk of frosts during seed filling (Robertson *et al.* 2004). In Australia, the change in yield with late sowing is variable, ranging from a loss of 10% per week to a gain of 4% per week with the mean response of 5% loss per week (Robertson *et al.* 1999). Simulation modelling by Farre *et al.* (2002) suggested that yield reduction can vary from 3.2% to 8.6% per week delay in sowing in high and low rainfall areas, respectively. Si and Walton (2004) concluded that early sowing would be essential for higher canola yields in Western Australia. Similarly (Gunasekera *et al.* 2006) in Western Australia found higher yield in canola and Indian mustard when it was sown early in low rainfall environments.

2.10.2. Cultivars

The ability to affect the final yield with different genotypes and/or environmental adaptation is well known. Canola has a wide range of spring cultivars from open-pollinated to triazine tolerant (TT) and Clearfield™ hybrids which show differences in biomass production and seed yield. Hybrid cultivars have higher yield potentials than conventional canola cultivars (Mahli *et al.* 2007). Brandt *et al.* (2007) found that hybrid cultivars yielded 12.5% more over the open pollinated on average mainly because they produced 17.6 % more biomass than open pollinated varieties. A common observation by farmers and consultants is that many hybrids show greater vegetative growth than open pollinated varieties but this difference in biomass is not always reflected in greater seed yield. However, there has been no critical evaluation of the patterns of growth and yield of hybrid varieties in South Australia.

The triazine tolerant (TT) herbicide resistance trait can restrict biomass and seed yield production in canola. Robertson *et al.* (2002b) found that the TT varieties had lower radiation use efficiency (RUE) and less biomass production with average 26% less yield than non TT cultivars. They also show that the TT trait has no effect on harvest index. Moreover they concluded that the TT trait has low early vigour which affects photosynthetic activity and slow in phenological development, hence varieties with the

TT trait produced lower yields than non TT types. However they did not examine differences in NUE and WUE. Gunasekera *et al.* (2006) found that mustard (*Brassica juncea*) has better average seed yield stability due to its tolerance to stressful conditions, better early vigour, shorter pre-anthesis phase and higher dry matter production than canola of similar maturity. Differences in early vigour between Indian mustard and canola was also reported by Hocking *et al.* (1997e). However, greater biomass production under water stress conditions in mustard may not be reflected in higher seed yield of mustard due to their lower harvest index. Higher dry matter production after anthesis in mustard compared with canola was also reported by Lewis and Thurling (1994). Different cultivars of oilseed brassica have different growth patterns but they all end up with a low harvest index (Table: 2). To explore future opportunities for the breeding and adaptation to various environmental conditions, there is a need to understand interactions between cultivars and various management practices.

Table 2: Difference between Harvest Index of various cultivars of oilseed brassica

	Spring type canola			
Harvest index (%)	Triazine tolerant	Non Triazine Tolerant	Mustard	References
	20-22	25-35.4	19.6-29.6	Robertson et al. 2002; Wright et al.1995

2.10.3. Balancing pre- and post-flowering water use

In ideal situations there should be correct balance between pre and post–flowering/anthesis water use of any crop (Doyle and Fischer 1979; McDonald 1992). Low water use during early development of crop (rosette to green-bud), either due to scarcity of available water and/or due to inability of the crop to use water present in soil, can hinder N uptake and hence the final yield of canola (Hocking 1987; Bernardi and Banks 1993). On the other hand, water stress during the post-flowering growth stages (flowering-seed filling) can reduce seed yield (Dreccer *et al.* 2000a). Water stress during the latter part of crop development can limit the number of flowers and pods and also prevent the translocation of N to seed, which results in lower nitrogen harvest index (NHI) and seed yield. Passioura (1976) and Fischer (1979) found a balance between of 70:30 in pre and post-anthesis water use in wheat crop under rainfed conditions. In South Australian Mediterranean environments, evaporation increases as the season progresses and the rainfall (amount and frequency) decreases considerably. So during post-flowering development crops mostly rely on the stored soil water, which is used much more efficiently than water used in the pre-flowering phase. Angus and Van Herwaarden (2001) found WUE of 6-7 kg ha^{-1}mm^{-1} for the pre-anthesis water use, as compared to 33 kg ha^{-1}mm^{-1} for post-anthesis water use. Norton and Wachsmann (2006) also showed the great value of a small amount of water during post flowering period for canola seed yield in the Victorian Wimmera region of Australia. Data from glasshouse experiments on wheat also support the above finding as seed yield improvements were noticed in treatments where plants were forced to save water for post-anthesis growth (Passioura 1976). Furthermore, it has been observed that improving evapotranspiration during the vegetative growth period was less effective in increasing seed yield in wheat than delaying the evapotranspiration of the same amount of water to the seed filling stage (Van Herwaarden *et al.* 1998). Approaches like cultivar selection, plant density, rate and timing of N application can be manipulated to restrict pre-flowering water use so that more water can be made available for post-flowering development. This water will not only help in improving the post-flowering photosynthesis but will also provide the time for translocation of reserves from the source to sink (seed) and can also improve the NUE (McDonald 1989).

2.10.4. Reducing soil evaporation

In the Mediterranean environment of South Australia, soil evaporation generally varies between 60-150 mm, which is typically 30% of total crop water use (evapotranspiration) with a potential to be as high as 50% (Angus and Van Herwaarden 2001). Soil evaporation can be altered by any crop management tool which involves the alteration of canopy cover through biomass production. Asseng *et al.* (2001) found that soil evaporation can be reduced by 18% - 27% on different soil types by adding N to a wheat crop. Some researchers have shown that total crop water use can be similar different treatments despite there being noticeable differences in growth and yield (Norton and Wachsmann 2006). They found that transpiration efficiency and the total water use did not change with N treatments. However, there was a significant difference between the N treatments in seed yield and growth from which they concluded that the application of N would have reduced soil evaporation by improving the early vigour of the crop, thereby making more water available for transpiration. This effect of N on partitioning of water use between E and T by improving the early vigour in different genotypes of wheat has been previously reported by (Richards and Lukacs 2002). Sadras (2003) revealed that soil evaporation would account for a substantial portion of evapotranspiration for seasons with high frequency of small rainfall events due to Stage 1 evaporation from soil. In case of heavier and less regular rainfall events, water percolation to deeper soil layers would reduce the amount of water available for soil evaporation; hence soil evaporation would be lower. In the former case, a more consistent relationship between seed yield, growth and water use can be seen.

Interception evaporation is defined as the evaporation from leaf surface and is generally not taken into account for field crops. However, from catchment studies it is recognised that it affects the efficiency of the catchment area (Calder 1986). Interception evaporation can be higher in a canopy with high leaf area which is usual in treatments with N application. Norton and Wachsmann (2006) argued that interception evaporation can increase the relative humidity in the crop canopy, which can reduce transpiration, and this could lead to around a 10% overestimation of transpiration values. However this concept needs further clarification as the effect of interception evaporation is not very clear yet.

2.10.5. Rate and delayed application of N on targeted growth stages

Oilseed brassica has a high demand for N, depending upon the length of growing season, soil type and target yield (Table: 3). Timing of N in relation to plant requirements at different growth stages is important to enhance yield, NUE as well as to reduce the cost of production. Time of N application in relation to growth stages can help to achieve higher efficiencies of inputs, prevent crop injury and improve profitability (CCC 2003).

Table 3: N requirements of canola in different parts of the world.

Location	Type	N requirement (kg ha^{-1})	Yield (t ha^{-1})	References
Canada	Spring	200	1.82	(Soper 1971; Nuttall *et al.* 1987; Bailey 1990, Karamanos *et al.*2007)
Alaska	Winter	160	2.12	(Lewis and Knight 1987)
UK	Spring	210	1.8	(Holmes and Ainsley 1977)
Egypt	Spring	213	2.5	(Ibrahim *et al.* 1989)
India	Spring	60-90	1.2	(Kumar and Chauhan 2005)
Argentina	Spring	150	----	(Chamorro *et al.* 2002)
Australia	Spring	50-90	1.3	(Gunasekera *et al.* 2006)
China	Spring	90-150	2.8	(Yang *et al.* 2006)

The response of oilseed brassica to timing of N application during the season is inconsistent (Barłóg and Grzebisz 2004b). Sieling *et al.* (1998b) reported that the effect of split application of N is closely related to the length of the crop growing season.

Nitrogen application in three splits generally performs well, with the application at budding stage showing clear benefits in terms of seed yield over a single application e.g. (Sieling *et al.* 1998b). Recently the effect of timing of N application on NUE and yield enhancement in winter canola was reported by Barłóg and Grzebisz (2004a). They found two and three splits of N application were equally effective.

In Europe, yield response to autumn applied N is low whereas faster crop growth in spring requires early N application to complement the slow soil mineralisation (Walker and Booth 2001). The benefit of N application in two splits, one in spring at the beginning of regrowth and the second at the beginning of shooting (stem elongation), was reported by Rathke *et al.* (2006). These authors also suggested that the split of the N application should be adjusted according to climatic conditions. A study on the timing of application in Iran reported a 26% higher yield when N was applied in three splits at sowing, start of flowering and 50% flowering than a single N application (Karamzadeh *et al*.2010). The physiological investigation of Barłóg and Grzebisz (2004a) shows that there are some critical stages in canola growth when high N nutrition is required for better yield formation. An early application at rosette stage of N leads to higher vegetative growth whereas delayed application up to inflorescence and/or at flowering assists yield formation with less vegetative growth (Rathke *et al.* 2006). Furthermore, delayed application until flowering of N in oilseed brassica enhances apparent use of N as well as physiological and agronomic efficiencies with the better uptake of N during reproductive growth. Barłóg and Grzebisz (2004b) found that the distribution pattern of the N application influenced N uptake rate, N mobilization and NUE.

There has been limited research on the effect of nitrogen management on NUE and WUE in areas with rainfall less than 450mm per annum such as the medium rainfall zone of South Australia. In the South Australian Mediterranean environment N is generally applied at 40-60 kg N ha^{-1} prior to sowing (Parker 2009). However it can be economically risky in dry conditions with variable spring rainfall (Potter 2009; Potter *et al.* 2009). Splitting N allows more time for decision making based upon better yield prediction, and growers can manage fertiliser N in response to water availability and crop demand in these environments (Sadras 2004; Potter 2009). However usually N rate and timing are selected arbitrarily rather than based on an understanding of the interactions between N supply and phenological development. Studies based on the plant N status at different growth stages showed the importance of N during the early developmental stages (rosette to green-bud) of canola (Hocking 1987; Bernardi and Banks 1993), where yield of oilseed rape remained source-limited during the seed filling stages (Dreccer *et al.* 2000a) and this limitation could be removed by adding N during seed filling. Moreover, a simulation study of Habekotté (1997) suggests improving the source and sink capacity simultaneously for raise the potential yield of winter canola. So strategies for N management need to be fine-tuned to provide N according to the crop’s need, which should improve NUE and assist in achieving high yield. Despite the importance of N nutrition to canola yields, there is surprisingly little local work on the effects of timing of N on biomass production, NUE and WUE.

2.11. Summary

Time of sowing, cultivar selection, water use and rate and timing of N application have been shown to play important roles in achieving higher yield, NUE and WUE of canola. Inappropriate management of these factors leads to poor NUE of canola and hence it will affect the water use and its efficiency which is probably a major cause of low yields in dryland farming. Without a good understanding of the limitations to WUE, achieving improvements in productivity through changes in agronomy or breeding will be difficult. Based on the review of the literature and experiences of local canola agronomists and farmers, there appears to be a mismatch between biomass production and water use and nitrogen management of crops is often poor. Despite extensive canola production in southern Australia, partly because of its value as a break crop, there are still important gaps in our knowledge about the limitations to productivity of canola. The main knowledge gaps are summarised as follows:

- Many hybrids show vigorous vegetative growth but with little yield benefit: how can we improve harvest index in canola? Is this related to the imbalance between pre-: post-anthesis crop water use?
- Canola has a high N requirement, but its management in rainfed systems has not received adequate attention. Is it possible to optimise N management for maximising water use and improved WUE?

Based upon the literature review and existing knowledge gaps the aim of the proposed work is to improve the productivity of canola in rainfed cropping systems by improving the N- water co-limitation and to improve the NUE and WUE with the specific aims of:

- To improve our understanding of the physiological limitations to yield of canola in rainfed cropping systems
- To describe and quantify the effects of genotype and important management practices on NUE and WUE.

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Chapter 3

3. Response of canola and mustard to nitrogen applications at key phenological growth stages in a Mediterranean environment

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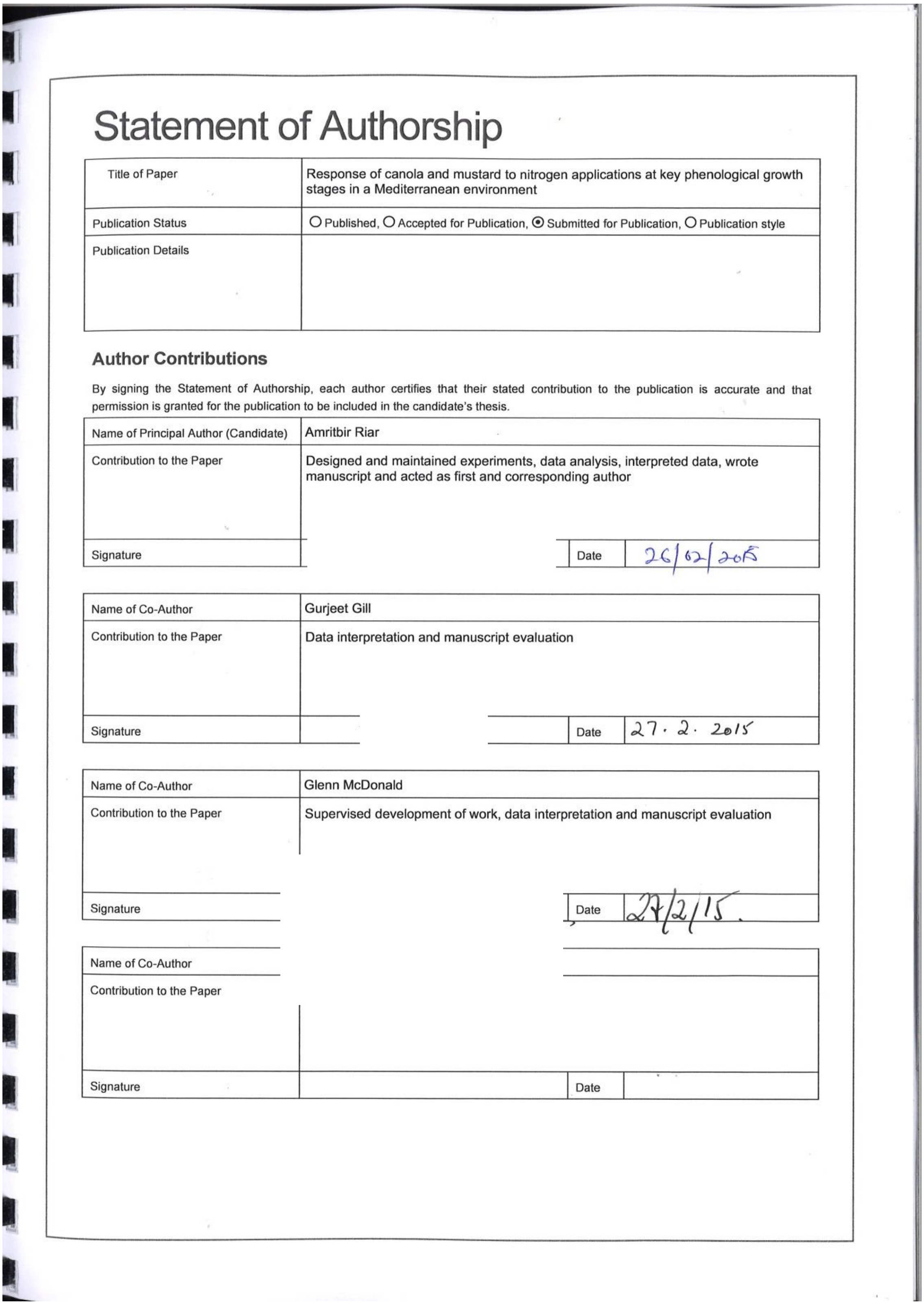

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Response of canola and mustard to nitrogen applications at key phenological growth stages in a Mediterranean environment

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3.1. Abstract

Water and nitrogen (N) are key factors limiting in canola production in southern Australia but the influence of interactions between N and water and on canola seed yield is still unclear. Altering the amount and the timing of N according to phenological development can be an effective way of managing variation in seasonal rainfall, which could improve seed yield. Consequently the aim of this study was to investigate the effect of N applications at targeted phenological stages on growth and yield of different canola and mustard cultivars. Field experiments with eight different N application strategies comprising two N rates (100 kg ha^{-1} and 200 kg ha^{-1}) and targeting growth stages (rosette, green bud, flowering, pod initiation and pod development) of four canola cultivars and two mustard cultivars were conducted at Roseworthy in South Australia over two growing seasons with contrasting rainfall. Mustard and canola performed similarly in the higher rainfall season but canola out-yielded mustard in the season with below-average rainfall. Seed yield of canola and mustard was found to be closely associated with total dry matter production and seeds m^{-2}. The yield penalty for triazine tolerant cultivars was lower in the season with dry spring than in the season with average rainfall. N improved the seed yield in both seasons. Seed yield of canola and mustard was highly responsive to the application of N at the rosette stage. Application of 100 kg ha^{-1} N at rosette stage produced yield equivalent to 85% of the maximum in canola and 94% of the maximum in mustard, which was achieved in non-limiting N treatment of 200 kg ha^{-1}. These results

suggest that increasing the sink capacity by improving the pre-flowering crop biomass has an important influence on seed yield of canola and mustard.

3.2. Introduction

Canola (*Brassica napus*) has become the most widely-grown broadleaf crop in Australia and its area in drier regions of southern-Australia has increased dramatically in the last decade. Mustard (*Brassica juncea*) is a quick developing crop, which has been often suggested as an alternative to canola in low rainfall areas. With the development of canola quality *B. juncea* that meets Australian oilseed industry standards, it is now possible to grow mustard commercially (Burton *et al.* 2008). However, the relative performance of canola and mustard in areas with medium and variable rainfall is still unresolved. Moreover, herbicide-tolerant canola systems can make canola a more attractive proposition for many growers. Triazine tolerant (TT) canola cultivars first became commercially available in 1993. The TT trait is usually associated with a yield penalty due to lower photosynthetic efficiency but TT canola cultivars are extensively cultivated due to their lower cost of production and compatibility with herbicide use and rotations (Robertson *et al.* 2002b). Imidazolinone herbicide tolerant (Clearfield®) canola cultivars are now becoming popular with growers due to their higher yield potential than TT cultivars. With all these options, canola has not only become a profitable crop in its own right, its contribution to weed control and as a disease break has made it an essential part of Australian farming systems.

Traditionally canola was grown after legume-based pastures to use high mineral nitrogen (N) in the soil and to break the cereal root disease cycle. However, with the intensification of the cropping system and increased popularity of canola, it is now often grown after cereals on soils with low N status which requires large application of N to achieve high yields. The introduction of hybrid cultivars with high early vigour, better weed control and increased use of N fertilisers have improved canola yields but there is still a large gap between actual and attainable yields (Lisson *et al.* 2007; Kebede *et al.* 2010). Nitrogen and water are the key factors limiting canola performance in Mediterranean environments and water availability has a large influence on the demand for and response to N. The availability of N depends on pre-sowing mineralization of N and within season mineralization of organic N, whereas water is always a limiting resource and its availability depends on the total rainfall during the fallow and growing season which vary seasonally. In Australia, Most of the research on canola adaptation and its interactions with N and water has been done in areas with more than 450mm annual rainfall where yield potential is high (Wright *et al.* 1995; Hocking *et al.* 1997e; Robertson *et al.* 2002b; Zhang *et al.* 2011). Limited research has been reported from areas with rainfall less than 450mm per annum such as the medium rainfall zone of South Australia. In these areas, N is generally applied at 40-60 kg N ha^{-1} prior to sowing (Parker 2009) whereas Potter *et al.* (2009) suggested that only 10 kg N ha^{-1} as pre-sowing is safe, when sowing early into warm and dry soils. It indicates that applying the entire N at the start of the season can be economically risky because of variable spring rainfall. Even in growing seasons with above-average rainfall, addition of all N at seeding could end up with low yield either due to N losses or less N availability than crop demand. It is often suggested that growers should manage fertiliser N in response to water availability and crop demand in these environments (Sadras 2004; Potter *et al.* 2009), but most of the time N rate and timing are selected arbitrarily rather than based on the understanding of the interactions between N supply and phenological development. Studies based on the plant N status at different growth stages showed the importance of N during the early developmental stages (rosette to green-bud) of canola (Hocking 1987; Bernardi and Banks 1993) whereas Dreccer *et al.* (2000a) argued that yield of oilseed rape remain source limited during the seed filling stages and found that this limitation can be removed by adding the N during seed filling. Moreover, based upon simulation study, Habekotté (1997) suggested to improve the source and sink capacity simultaneously for to increase the potential yield of winter canola. Thus in this study our focus is to investigate, if targeting a specific phenological growth stage for N application can improve the yield of canola and mustard by overcoming the limitations of source and sink capacity for yield determent factors in canola and mustard under Mediterranean environment conditions.

3.3. Material and methods

3.3.1. Site description

Field studies were undertaken on the Roseworthy farm of the University of Adelaide (latitude 34.53 °S; longitude 138.72 °E), South Australia during the 2011 and 2012 growing seasons. The long term annual average rainfall for Roseworthy is 440mm with the growing season average rainfall (defined in South Australia as rainfall from April to October; (French and Schultz 1984)) of 329 mm.

The main soil type of the sites was a Chromosol with an alkaline trend down the profile (Isbell 2002). In order to estimate soil moisture and nitrate-N up to a depth of 100 cm, soil cores were taken 2 days prior to sowing in both seasons by using a 4 cm hydraulic core. Soil samples were taken at five depths from across the sites at sowing, bulked, dried at 40°C and sieved (<2mm) for analysis by a commercial laboratory (CSBP, Perth Western Australia). Total amount of the mineral-N (ammonium + nitrate) in 0-100 cm layer was 77 kg ha^{-1} and 71 kg ha^{-1} in 2011 and 2012 respectively. Detailed soil characteristics of the experimental sites are given in Table: 1.

3.3.2. Experimental design

The experiments compared the growth and yield of different canola and mustard cultivars (Table: 2), grown under different N application strategies comprising three N rates (0, 100 and 200 kg N/ha as granular urea; 46% N) and different timings of application

(Table: 3). Canola and mustard varieties with similar maturities but with differences in early vigour were selected to represent varieties commonly grown in the region. They included open pollinated (OP), hybrid, conventional, TT and Clearfield varieties. In 2011 the treatments were arranged in a split-split plot design with three replications with time of sowing as the whole-plots, canola and mustard cultivar as the sub-plots and N treatments as sub-sub-plots. In 2012 only one time of sowing was used so the treatments were arranged in split plot design with cultivars in whole-plots and N treatments in sub-plots with three replicates.

Table 1: Soil characteristics of each site used during 2011 and 2012. Analyses were conducted by CSBP Soil and Plant Analysis Laboratories, Perth WA using the methods described in by Rayment and Lyons (2011).

Year	Layer (cm)	Ammonium N[1] (mg kg^{-1})	Nitrate N[2] (mg kg^{-1})	Colwell P[3] (mg kg^{-1})	Colwell K[4] (mg kg^{-1})	Sulphur[5] (mg kg^{-1})	Organic C[6] (%)	Conductivity[7] (dS/m)	pH level H2O (pH)[8]	Boron[9] (mg kg^{-1})	Calcium carbonate[10] (%)
2011	0-20	7.0	4.0	23.0	580	7.2	0.99	0.211	7.5	2.07	9.14
	20-40	3.0	2.0	7.0	220	7.2	0.57	0.170	8.2	2.39	30.40
	40-60	2.0	1.0	10.0	222	18.0	0.32	0.230	8.1	6.18	47.84
	60-80	2.0	2.0	3.0	424	56.8	0.20	0.464	8.5	17.03	40.80
	80-100	1.0	5.0	<2.0	543	66.7	0.15	0.404	8.7	25.50	37.44
2012	0-20	12.7	10	56.7	509	20.1	1.21	0.300	7.7	1.89	0.98
	20-40	5.3	4.0	13.7	192	12.5	0.66	0.200	8.6	3.25	20.39
	40-60	4.0	1.6	9.7	132	16.8	0.34	0.210	8.8	4.21	45.76
	60-80	3.0	1.7	5.0	179	29.6	0.25	0.428	9.1	9.62	53.53
	80-100	2.3	2.0	3.0	276	55.0	0.19	0.622	9.3	13.19	52.49

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[1] Method No. 7C2b pp 130
[2] Method No. 7C2b pp 130
[3] Method No. 9B &18A1 pp 162 & 385
[4] Method No. 9B &18A1 pp 162 & 385
[5] Method No. 10D1 pp 223
[6] Method No. 6A1 pp 68
[7] Method No. 4A1, 4B3 & 3A1 pp 38 & 20
[8] Method No. 4A1, 4B3 & 3A1 pp 38 & 20
[9] Method No. 12C1 & 12D1 pp 244
[10] Method No. 19B2 pp 420

Table 2: Details of the cultivars used for experiments during 2011 and 2012

Cultivars	Type	Species	Origin	Maturity
AV Garnet	Open pollinated (OP)	Canola	Australia	Mid
FighterTT	Open pollinated (OP)	Canola	Australia	Early- mid early
Hyola555TT	Hybrid	Canola	Australia	Mid-Mid early
Hyola575CL	Hybrid	Canola	Australia	Mid-Mid early
Oasis CL	Open pollinated (OP)	Mustard	Australia	Early
Varuna	Open pollinated (OP)	Mustard	India	Early

Nitrogen treatments were designed to generate a range of biomass and canopy size (Tables: 3) and targeted at specific growth stages. A control treatment with no N and a high N control in which a total of 200kgN ha^{-1} was applied in five equal splits (rosette (GS30), green-bud (GS51), start of flowering (GS61), start of pod filling (GS67) and 10% pod maturity (GS71)) was used to maintain a steady supply of N throughout the season. These controls were designed to provide a boundary function of crop response to N in both years. All other treatments were designed to examine the effects of N supply at a specific growth stage. The growth stages were recorded by using the BBCH canola scale (Lancashire *et al.* 1991). Nitrogen was spread by hand close to the desired growth stage either when the soil was wet or if rainfall was forecast within 24h after application. On an average, there was 13.2mm and 9.9mm rainfall in a week time after N applications in 2011 and 2012 respectively.

Table 3: Details of N rate, number of split applications and growth stages for N application used during 2011 and 2012

Year	Rate	Splits	Targeted Growth Stages (BBCH scale)
2011+2012	0	0	0
2011	100	3	30,63,71
2011	100	2	51,67
2011	100	2	63,71
2012	100	1	30
2012	100	1	51
2012	100	1	63
2012	100	5	30,51,63,67,71
2011+2012	200	5	30,51,63,67,71

3.3.3. Crop management

The trials were sown in no-tillage system with a cone seeder with knife point drill and press wheels at a depth of 25mm. Plots were 10 metre long consisting 6 rows with 250 mm inter-row width. Basal fertilisers were 10 kg N ha^{-1} and 11 kg P ha^{-1} as diammonium phosphate (DAP) applied at sowing and 100 kg S ha^{-1} as a pre-plant gypsum application. Seeding rates were adjusted for each cultivar based on their seed weight and germination test to achieve a plant establishment of 35 plants m^{-2}. Plant numbers were counted after crop establishment and it showed that on average there was 84% and 82 % establishment in 2011 and 2012, respectively (In 2011, range between varieties 73% to 98% and range between varieties 79% to 93% in 2012, difference in establishment was statistically non-significant).Weeds were controlled by a pre sowing application of glyphosate (2.8 L ha^{-1}) and by hand weeding in crop when required. To avoid any early damage by insects, chloropyrifos (Lorsban 700 ml ha^{-1}) was sprayed two days after sowing. Slugs and mouse damage were managed by applying snail bait (5 kg ha^{-1}) and bromadiolone (Mouse off @ 2-4 kg ha^{-1}) when necessary. Overall weed and disease incidence was minimal during both years.

3.3.4. Measurements and sampling

Crop dry matter was measured at rosette (GS30), green-bud (GS51), 50% flowering (GS55) and 50% pod development (GS75) and maturity (GS 99). For measurements between GS30 and GS75 destructive samples from two rows of 50cm length (0.25 m^2) were taken. The samples were dried in an oven at 80 °C for 48 hours. Canopy light interception measurements were taken immediately after/before the dry matter sampling during rosette (GS30), green-bud (GS51), 50% flowering (GS55) and 50% pod development (GS75) from 5 random locations within a plot using an AccuPar-ceptometer (Delta-T Devices Ltd.). The radiation was measured in the photosynthetically active radiation (PAR) range (400–700 nm) and measurements were taken on clear cloudless days within 2 h either side of solar noon. For each measurement, five above-canopy reading and five below-canopy readings were taken. Seasonal Radiation use efficiency (RUE) was calculated for each treatment by linear regression of sequential crop biomass measurements between the rosette (GS31) and pod-development (GS75) against intercepted PAR (Mendham *et al.* 1981). At maturity plants from two rows of 50cm length (0.25 m^2) equivalent to a half metre row were taken from each plot to estimate pod numbers per plant, seeds per pod, and harvest index (HI). The number of pods and seeds were measured on a subsample of a quarter of the inflorescence weight using the mass of four plants and then converted to pod m^{-2} and seeds m^{-2} according to the plant density estimated in each plot. Harvest index was estimated as the ratio of seed weight to total shoot weight of the subsample. Total dry matter at maturity was calculated based on seed yield and HI. Seeds per pod were estimated by counting seeds from a sample of 25 pods from 5 different positions from the plant inflorescence, including main raceme and sub-branches. Mean seed weight was estimated from the weight of 1000 seeds.

3.3.5. Statistics

The data obtained from the experiment were analysed by analysis of variance (ANOVA) using the GenStat statistical analysis software (15th edition). Orthogonal contrasts were used to compare the different groups of cultivars and N treatment based on single degree of freedom comparisons (VSN 2013). For other statistical comparisons, the least significant difference (LSD) at 5% level of probability (P=0.05) was used to compare the treatments unless otherwise stated.

3.4. Results

3.4.1. Seasonal conditions

Growing season (April-October) rainfall was similar in both years (232 mm in 2011 and 220 mm in 2012) but the annual rainfall (January-December) was 102 mm lower in 2012 (292 mm) than 2011 (394 mm). In 2011, the season had a wet start with above-average rainfall in February and March and an average finish to the season whereas 2012 had a dry finish due to the below-average rainfall in September and October (Fig:1a). The maximum temperatures for the growing seasons in both years were higher than the long term mean temperatures (Fig: 1b) whereas the mean minimum temperatures for the site were lower than the long term mean minimum temperature.

3.4.2. Crop development

The N treatments (rate and timing) did not affect the phenological development of canola and mustard in either year. Mustard flowered earlier and for a longer time than canola in both years. In 2011, delaying sowing by 27days reduced the growing period by 17days (range 12-19 days) without affecting the pre-flowering and post flowering period (Fig: 2). Delayed sowing reduced the flowering period by 18 days (38%) in canola and by 24 days (36%) in mustard. Among canola cultivars, reduction in flowering period by delayed sowing was larger for TT cultivars (24 days i.e. 52%) than non-TT cultivars (13 days i.e. 25%). Comparing May sown crops of 2011(6th May) and 2012 (17th May) revealed that length of the pre-flowering and flowering periods was similar in both years for all Australian cultivars (Canola and Oasis). For Varuna (Indian mustard) pre-flowering and flowering period were longer in 2012 than 2011 by 11 and 7 days respectively. The post-flowering period was shorter in 2012 than 2011 for all cultivars. In canola, post-flowering period in TT varieties was shorter by 14 days (32%) and by 16 days (41%) in non-TT varieties. In mustard, post-flowering period was shorter in Oasis by 14 days (41%) and 26 days (59%) in Varuna. At each sowing time, all canola and mustard cultivars matured within a one week window.

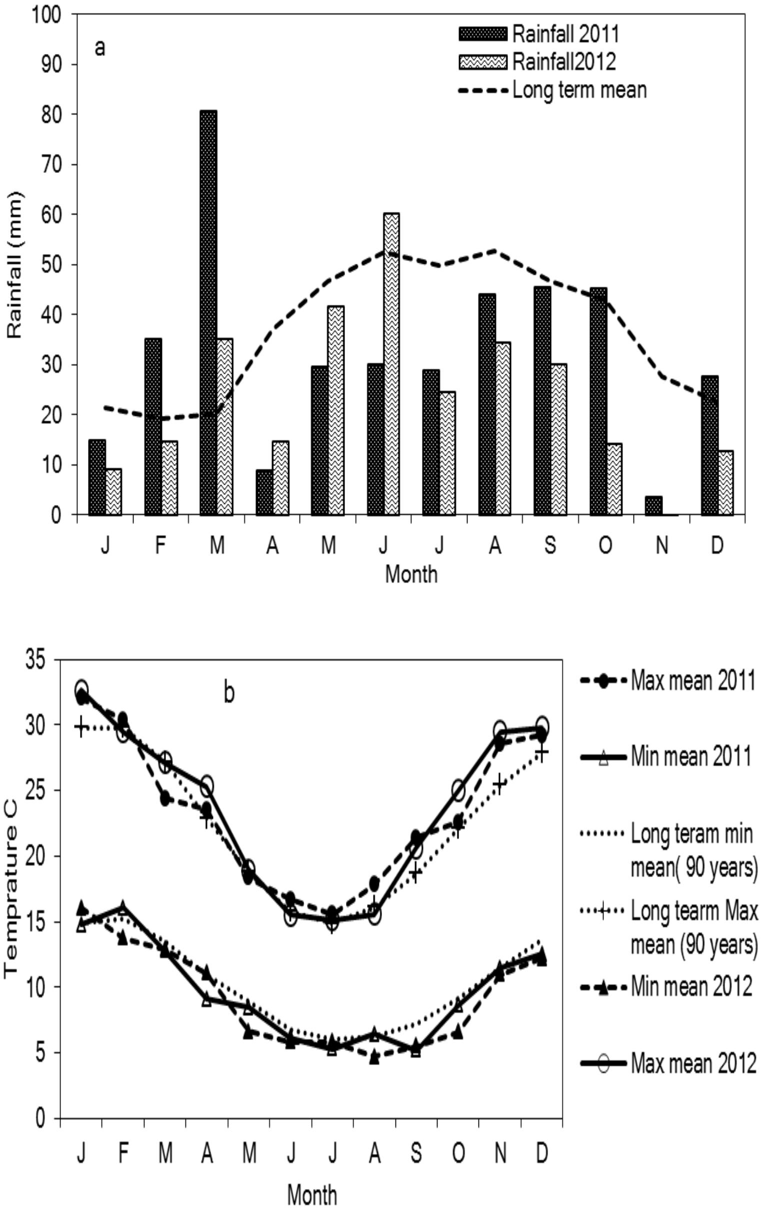

Figure 1: Monthly rainfall, maximum and minimum temperature during 2011 and 2012 with long term means of 90 years for Roseworthy.

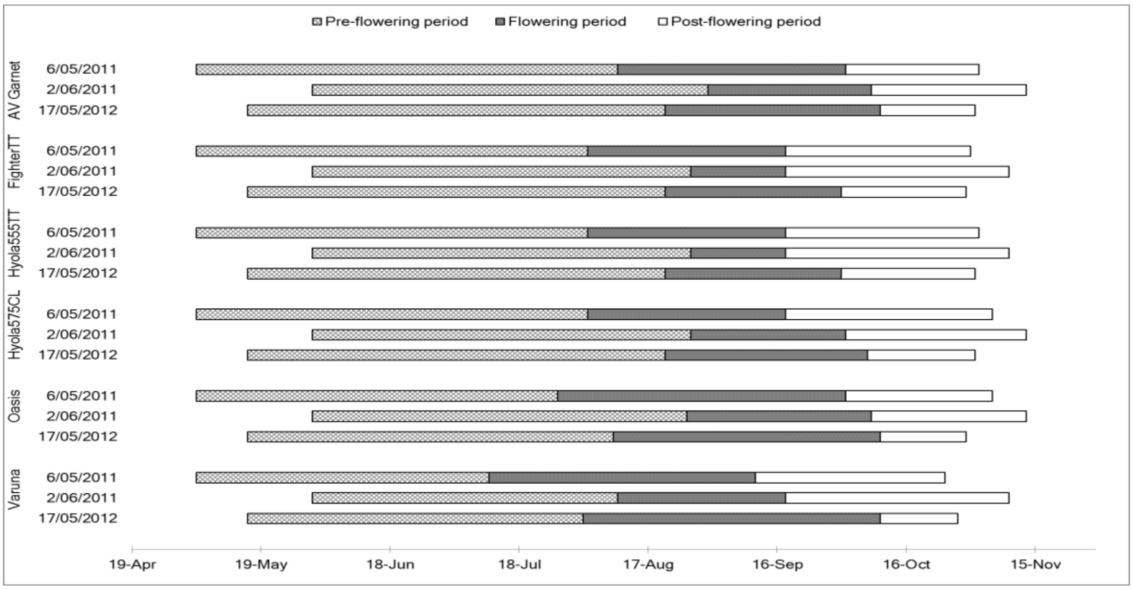

Figure 2: The pre-flowering, flowering period and post flowering period of different cultivars at different time of sowings during 2011 and 2012

3.4.3. Dry matter accumulation

In 2011 biomass continued to increase up to maturity whereas in 2012 biomass production was considerably lower and slowed soon after flowering (Fig: 3). In 2011, mustard produced the highest biomass followed by non-TT canola and TT canola (Fig: 3). In 2012, mustard produced more biomass than canola up to flowering but both crop species produced similar biomass during the post-flowering period. All canola varieties produced similar biomass up to pod-development (GS75) in 2012 but at maturity higher biomass was observed in non-TT cultivars. There was no difference in biomass production between OP and hybrid cultivars at most of the growth stages in both years except at flowering and maturity in 2011, when hybrid cultivars produce more biomass than OP cultivars. Varuna produced more biomass than Oasis in both years except at the pod-development stage, where both were statistically similar in 2012.

In 2011, 200 kg N ha^{-1} in five identical splits produced higher crop biomass than 100 kg N ha^{-1} (Table: 4). Applying 100 kg N ha^{-1} in three splits produced more biomass than the same amount of N applied in two identical splits. In 2012, there was no difference in biomass production between the different rates of N application up to pod development stage but at maturity 200 kg N ha^{-1} in five equal split applications produced more biomass with continuous growth while growth stopped in 100 kg N ha^{-1} treatments (Table: 4). There was no difference between split and single applications of 100 kg N ha^{-1} in terms of shoot DM. Delaying the application of N beyond the rosette stage produced less biomass than its application at the rosette stage (P=0.10).

Table 4: Dry matter accumulation and RUE with different N application during different growth stages during 2011 and 2012

			Dry matter (kg ha^{-1})				
Year	N Rate	Targeted GS for N	Rosette	Green-bud	Flowering	Pod-development	Maturity (TDM)
2011	0		318	1316	3035	5714	6582
	100	30,63, 71	294	1693	3876	7023	8353
	100	51,67	326	1479	3542	6444	7468
	100	63,71	292	1271	3099	5769	6616
	200	30,51,63,67,71	326	1717	4260	7289	8799
	lsd		72	311	498	558	904
2012	0	0	120	1132	2571	3029	2606
	100	30	81	1647	3669	3434	3349
	100	51	85	1161	3405	3102	2850
	100	63	119	1153	2471	3184	2922
	100	30,51,63,67,71	89	1238	2968	3333	2978
	200	30,51,63,67,71	88	1219	3116	3087	4025
	Lsd		44	401	677	550	618

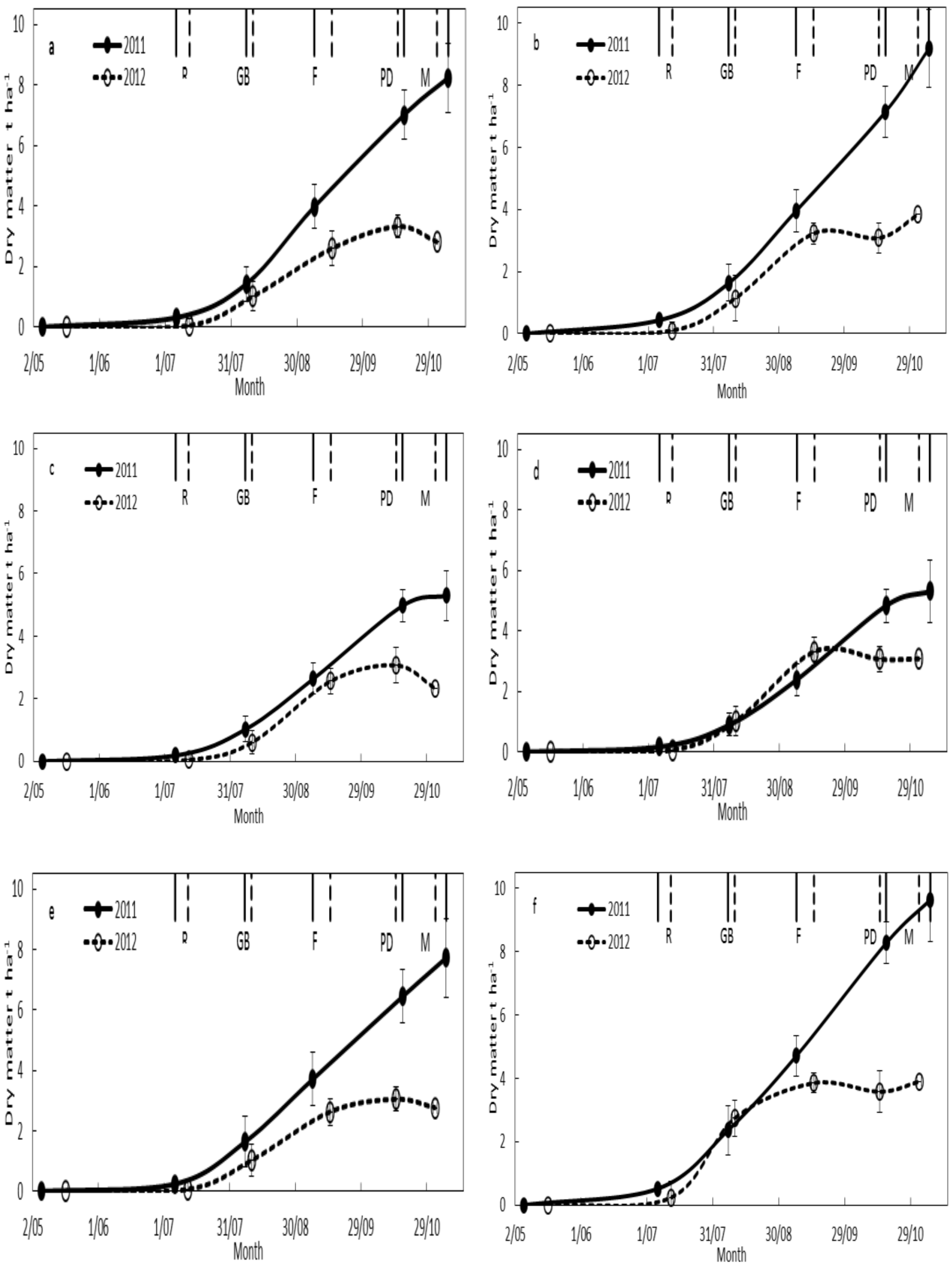

Figure 3: Dry matter accumulation of cultivars during the 2011 and 2012growing seasons. (a)AV Garnet (b) Hyola575CL (c) Fighter TT (d) Hyola555TT (e) Oasis (f) Varuna

3.4.4. Radiation use efficiency (RUE)

RUE of cultivars varied between the two seasons. In early sown (May) crop of 2011 RUE varied with cultivars e.g. RUE of Varuna, Hyola575CL, AV Garnet and Oasis was higher than FighterTT and Hyola555TT (Table: 5a). But in late sown (June) crop, canola had higher RUE than mustard. Orthogonal contrasts revealed that Mustard had higher RUE than canola when sown early but canola had higher RUE when sown late in season. Among early sown canola cultivars non-TT and hybrid cultivars had higher RUE than TT and OP cultivars.RUE was similar among cultivars during 2012 E (Table 5a). Adding N improved the RUE during early sowing of 2011 whereas it had no effect in late sown crop of 2011 and timely sown crop of 2012. (Table: 5b)

Table 5a: Seasonal RUE of different cultivars with different N application on targated growth stages during 2011 and 2012.

	2011				2012	
	TOS1		TOS2		TOS1	
Cultivar	Average RUE ± s.e (g MJ^{-1})	r	Average RUE ± s.e (g MJ^{-1})	r	Average RUE ± s.e (g MJ^{-1})	r
Av Garnet	3.31 ± 0.59*	0.97	2.20 ± 0.24*	0.99	1.22 ± 0.58	0.83
FighterTT	2.07 ± 0.76	0.89	1.30 ± 0.16*	0.99	1.29 ± 0.50	0.88
Hyola555TT	1.63 ± 0.39	0.95	1.31 ± 0.27*	0.96	1.23 ± 0.85	0.72
Hyola575CL	3.31 ± 0.59*	0.97	2.23 ± 0.05**	1.00	1.10 ± 0.82	0.69
Oasis	3.10 ± 0.41*	0.98	1.96 ± 0.51	0.94	1.31 ± 0.68	0.81
Varnua	3.46 ± 0.62*	0.97	3.04 ± 0.79	0.94	1.06 ± 1.00	0.60
Cultivar Groups						
Canola	2.48 ± 0.59	0.95	1.76 ± 0.22*	0.98	1.25 ± 0.68	0.79
Mustard	3.27 ± 0.51*	0.98	2.49 ± 0.63	0.94	1.27 ± 0.88	0.71
TT	1.84 ± 0.54	0.92	1.31 ± 0.21*	0.98	1.31 ± 0.67	0.81
non-TT	3.31 ± 0.59*	0.97	2.25 ± 0.28*	0.98	1.19 ± 0.69	0.77
OP	2.26 ± 0.72	0.93	1.74 ± 0.16**	0.99	1.26 ± 0.54	0.86
Hy	2.32 ± 0.49*	0.96	1.75 ± 0.32*	0.97	1.16 ± 0.84	0.70

Table 5b: Seasonal RUE of different N application on targated growth stages during 2011 and 2012

Year	TOS	Rate	Targeted GS for N	Average RUE ± s.e (g MJ^{-1})	r
2011	1	0		2.67 ± 0.67	0.94
		100	30,63,71	2.96 ± 0.59*	0.96
		100	51,67	2.67 ± 0.50*	0.97
		100	63,71	2.46 ± 0.54*	0.95
		200	30,51,63,67,71	2.87 ± 0.53*	0.97
2011	2	0		1.97 ± 0.33*	0.97
		100	30,63,71	1.99 ± 0.35*	0.97
		100	51,67	1.84 ± 0.30*	0.98
		100	63,71	1.84 ± 0.23*	0.98
		200	30,51,63,67,71	2.33 ± 0.45*	0.96
2012	1	0		1.18 ± 0.99	0.65
		100	30	1.21 ± 0.75	0.75
		100	51	1.46 ± 0.60	0.86
		100	63	1.38 ± 0.72	0.81
		100	30,51,63,67,71	1.20 ± 0.70	0.77
		200	30,51,63,67,71	1.16 ± 0.70	0.76

3.4.5. Total dry matter (TDM), Seed yield and HI

Total dry matter production was higher in mustard than canola in 2011, which was not reflected in seed yield due to higher HI of canola (Table: 6). Total dry matter produced in 2012 was lower than in 2011 but canola and mustard produced similar dry matter in 2012. However, mustard produced lower seed yield because of the greater HI of canola than mustard. In both years TT cultivars produced less biomass and seed yield with little difference in HI than non-TT cultivars (Table: 6). OP cultivars produced similar seed yield as the hybrid cultivars in 2011 due to their higher HI, but in 2012, lower seed yield and biomass production was observed in OP cultivars as HI for both groups was similar (Table: 6). Varuna mustard produced more dry matter at maturity than Oasis in both years but it was not reflected in differences in seed yield as the HI of Oasis was significantly higher than Varuna (Table: 7). Across all varieties, there was a strong correlation between seed yield and total biomass at maturity but not with HI in both years (Table: 8).

Nitrogen improved dry matter production and seed yield of canola and mustard (Table 4, 9 and 10). Higher seed yield and TDM was produced with 200 kg N ha^{-1} in five equal splits as compared to all treatments that received 100 kg N ha^{-1} in both years. Application of 200 kg N ha^{-1} did not affect HI in 2011 but in 2012, the highest N rate caused a significant reduction in HI as compared to 100 kg N/ha (Table: 10). In 2011, when 100 kg N ha^{-1} was applied in three splits, seed yield, TDM and HI ($P<0.10$) improved relative to the same amount of N applied in two splits. There was higher TDM ($P<0.10$) when 100kg N ha^{-1} N was applied at green-bud than at flowering in two equal splits but it was not reflected in seed yield and HI.

Table 6: Orthogonal comparisons for total dry matter, seed yield, HI and yield components between different groups of canola and mustard for 2011 and 2012

		TDM (kg ha^{-1})		GY (kg ha^{-1})		HI		Seeds m^{-2} (x10^{-3})		1000 seed weight (g)		Seeds Pod^{-1}	
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Canola vs Mustard													
	Canola	7006	3016	1855	1012	0.27	0.34	53.6	36.1	3.5	2.9	16.7	15.8
	Mustard	8680	3334	1977	855	0.23	0.27	54.9	28.8	3.8	3.2	13.5	13.5
	Prob	0.001	NS	NS	0.036	<.001	<.001	NS	0.014	<.001	0.021	<.001	NS
TT vs Non TT													
	TT	5302	2704	1467	887	0.27	0.34	42.6	31.5	3.5	2.9	17.2	16.2
	Non TT	8710	3328	2243	1136	0.26	0.34	64.6	40.7	3.5	2.9	16.1	15.3
	Prob	<.001	0.017	<.001	0.008	0.058	NS	<.001	0.009	NS	NS	0.044	NS
OP vs Hybrid													
	OP	6766	2572	1887	885	0.28	0.35	54.3	30.1	3.5	3.1	16.0	14.0
	Hybrid	7245.5	3460	1823	1138	0.25	0.33	52.9	42.1	3.4	2.7	17.3	17.5
	Prob	NS	0.002	NS	0.007	0.01	NS	NS	0.002	NS	0.012	0.025	0.085

Table 7: Effect of cultivars on seed yield, total dry matter, HI and yield components for 2011 and 2012

Cultivar	TDM (kg ha^{-1})		GY (kg ha^{-1})		HI		Seeds m^{-2} (x10^{-3})		1000 seed weight (g)		Seeds Pod^{-1}	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
AV Garnet	8235	2816	2326	1021	0.28	0.36	68.5	36.0	3.4	2.9	16.4	13.1
FighterTT	5297	2327	1447	749	0.27	0.34	40.2	24.1	3.6	3.2	15.6	15.0
Hyola 555TT	5306	3080	1486	1025	0.28	0.34	45.0	38.9	3.3	2.7	18.9	17.5
Hyola 575CL	9185	3840	2160	1251	0.23	0.33	60.8	45.3	3.6	2.8	15.7	17.5
Oasis	7724	2769	1840	822	0.24	0.30	63.8	33.5	2.9	2.6	12.6	11.4
Varnua	9635	3899	2114	888	0.22	0.23	46.1	24.1	4.6	3.8	14.3	15.5
lsd 0.05	1513	688	304	236	0.02	0.03	8.9	8.9	0.1	0.3	1.6	NS

Table 8:Correlation coefficients between seed yield, yield components, dry matter at various growth stages and HI including all N treatments for canola and mustard cultivars grown at Roseworthy during 2011 and 2012.(n= 20 and 24 for canola, 10 and 12 for mustard during 2011 and 2012 respectively).

	Canola					Mustard				
	GY	Pods m^{-2}	Seeds m^{-2}	Seeds Pod^{-1}	Seed weight	GY	Pods m^{-2}	Seeds m^{-2}	Seeds Pod^{-1}	Seed weight
2011										
DM_Ro	0.58**	0.28	0.53*	-0.33	0.28	0.27	-0.54	-0.62	0.63	0.86**
DM_GB	0.83***	0.40	0.78***	-0.34	0.26	0.37	-0.30	-0.29	0.35	0.67*
DM_F	0.93***	0.48*	0.90***	-0.41	0.13	0.89***	0.07	0.2	0.27	0.61
DM_PD	0.94***	0.42	0.91***	-0.39	0.14	0.82**	-0.09	0.02	0.35	0.75*
DM_M	0.92***	0.44	0.88***	-0.39	0.18	0.84**	-0.06	0.08	0.37	0.70*
HI	0.02	0.16	0.09	0.27	-0.37	0.452	0.82**	0.76*	-0.41	-0.46
2012										
DM_Ro	0.54**	0.05	0.54**	0.28	-0.44*	-0.06	-0.77*	-0.67*	0.44	0.82**
DM_GB	0.76***	-0.06	0.73***	0.38	-0.53**	0.17	-0.66*	-0.41	0.62*	0.66*
DM_F	0.38	-0.01	0.43*	0.38	-0.51*	0.63*	-0.32	-0.03	0.02	0.58*
DM_PD	0.04	-0.07	0.002	0.14	0.11	-0.003	-0.46	-0.25	0.17	0.34
DM_M	0.94***	0.06	0.92***	0.45*	-0.63**	0.79**	-0.18	-0.10	-0.07	0.55
HI	-0.18	-0.1	-0.19	-0.34	0.25	0.00	0.66*	0.63*	-0.26	-0.83***

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 9: Effect of N on seed yield, total dry matter, HI and yield component for 2011 and 2012

Year	Rate	Targeted GS for N	GY (kg ha^{-1})	HI	Seeds m^{-2} (x10^{-3})	1000 seed weight (g)	Seeds Pod^{-1}
2011	0		1448	0.23	41.3	3.5	16.2
	100	30,63, 71	2039	0.25	57.2	3.6	16.5
	100	51,67	1893	0.26	53.8	3.6	15.1
	100	63,71	1708	0.27	48.8	3.6	15.3
	200	30,51,63,67,71	2388	0.27	69.2	3.5	14.9
	lsd		224	0.01	7.1	NS	NS
2012	0	0	766	0.31	26.9	3.1	15.4
	100	30	1078	0.33	40.3	2.8	16.8
	100	51	863	0.32	29.3	3.0	13.9
	100	63	963	0.33	32.3	3.1	15.1
	100	30,51,63,67,71	942	0.32	33.4	2.9	13.7
	200	30,51,63,67,71	1142	0.29	39.8	3.0	15.1
	lsd		154	0.03	6.3	NS	NS

Table 10: Orthogonal comparisons for total dry matter, seed yield, HI and yield components between different N treatments for 2011 and 2012

Year	Orthogonal comparisons groups for N treatments	GY (kg ha^{-1})	HI	Seeds m^{-2} ($x10^{-3}$)	1000 seed weight (g)	Seeds Pod^{-1}
2011	N Treatments	<.001	<.001	<.001	NS	NS
	N vs no-N	<.001	<.001	<.001	NS	NS
	100 kg vs 200 kg	<.001	0.075	<.001	NS	NS
	100 kg in 2 splits vs in 3 splits	0.017	0.079	0.057	NS	0.04
	100 kg at Inflorescence vs at Flowering	NS	NS	NS	NS	NS
2012	N Treatments	<.001	0.039	NS	NS	NS
	N vs no-N	<.001	NS	0.001	NS	NS
	100 kg vs 200 kg	0.004	0.003	0.017	NS	NS
	100 kg single vs 5 split	NS	NS	NS	NS	NS
	100 at Rosette vs 100 at Inflorescence & Flowering	0.016	NS	<.001	0.038	NS
	100 kg at Inflorescence vs at Flowering	NS	NS	NS	NS	NS

3.4.6. Sink development (pods m-2, seeds m-2, seeds pod-1 and 1000 seed weight)

Cultivar × N interactions were observed in both years for pods m^{-2} (supplementary data), which showed non-significant correlation with total dry matter at maturity than seeds m^{-2} (Table: 8). In 2011, mustard and canola had similar seeds m^{-2} but mustard had fewer seeds per pod with higher seed weight (Table: 6) whereas in 2012, mustard had fewer seeds m^{-2} with a similar number of seeds pod^{-1} but higher seed weight than canola. The TT cultivars had a lower number of seed m^{-2} with similar seed weight and seeds pod^{-1} to non-TT in both years (Table: 6). Hybrid cultivars had a similar number of seeds m^{-2} as OP cultivars in 2011 but hybrids had more seeds m^{-2} in 2012. Seed weight of OP cultivars tended to be higher than hybrid cultivars but the effect was only significant in 2012 whereas hybrid cultivars had more seeds per pod in both years ($P < 0.10$ in 2012). Oasis produced more seeds m^{-2} than Varuna whereas Varuna had more seeds per pod and higher seed weight than Oasis (Table: 7).

In 2011, the rate of N increased the number of seeds m^{-2} (Table: 10). Application of 100 kg N ha^{-1} in 3 splits improved seeds m^{-2} ($P=0.10$) and seeds pod^{-1} as compared to the same amount of N applied in two split applications. All other treatments did not differ significantly from each other for seeds m^{-2}, seeds pod^{-1} and seed weight (Table: 9). In 2012, N improved the seeds m^{-2} but not seeds pod^{-1} and seed weight (Table: 10). Application of 200 kg N ha^{-1} in 5 splits produced more seeds m^{-2} than 100 kg N ha^{-1} applied at the green-bud or at flowering but not when it was applied at the rosette stage. The delayed application of 100 kg N ha^{-1} at green-bud or at flowering improved seed weight but decreased seeds m^{-2} over the earlier application at rosette stage.

3.5. Discussion

3.5.1. Canola and mustard in relation to seasonal conditions

In this study it became clear that total dry matter at maturity was the main determinant of canola and mustard seed yield. Growth of canola and mustard is characterised by leaf shedding during flowering, podding and seed fill, which often causes a reduction in biomass production in spring. However in 2011, the mild and wet growing conditions promoted continued biomass production. On an average, canola and mustard gained 54% and 52% biomass after flowering in 2011 respectively, whereas this gain was only 3% in 2012 for both canola and mustard. These alterations in growth pattern were reflected in a weaker relationship between seed yield and dry matter in 2012 than 2011 and indicate the importance of dry matter accumulation after flowering. Low dry matter accumulation after flowering was mirrored in 54% and 43% lower yield for canola and mustard respectively in 2012 as compared to 2011. Interestingly, mustard produced 6% more yield and 20% higher biomass at maturity than canola in 2011. In the drier conditions of 2012, mustard again produced 10% more biomass than canola but its seed yield was 21% lower than canola. These results indicate the superior ability of mustard to develop a bigger sink than canola due to earlier and longer flowering even under dry conditions but it was unable to convert that sink into superior seed yield in 2012. The higher biomass production of mustard in 2011 was in line with several studies (Lewis and Thurling 1994; Hocking *et al.* 1997e; Gan *et al.* 2004).However, the results from 2012 clearly contradict previous research. There was very little increase in crop DM after flowering in 2012 which would have prevented conversion of extra sink in mustard to seed yield. The lower seeds m^{-2} of mustard was the main cause of lower seed yield of mustard than canola during the drier growing season in 2012. In some areas canola has been shown to suffer greater pre-flowering DM loss due to leaf shedding than mustard and in such situations mustard can out yield canola (Hocking *et al.* 1997e). However in this study, there was a large gain in dry matter during the post-flowering period in 2011. Even in 2012, crop DM was relatively stable from flowering to maturity in canola and mustard cultivars. Statistical analysis showed superior ability of canola to withstand post-flowering stresses in water limited environments.

The yield penalty of TT cultivars compared to non-TT canola varies with environmental conditions and has been reported previously (Beversdorf *et al.* 1988; Robertson *et al.* 2002b). Usually**,** yield differences among TT and other cultivars tend to be small in low rainfall areas but can be quite large in high rainfall areas (Robertson *et al.* 2002b). In this study undertaken over two contrasting seasons, this trend was confirmed: the yield penalty in TT cultivars was lower in the drier 2012 season than in 2011.TT cultivars produced 39% less DM at maturity and 35% less seed yield than the average of non-TT cultivars in 2011 and the penalty for dry matter in TT cultivars was 19% and 22% for seed yield during the drier season of 2012. In both years TT cultivars produced fewer seeds m^{-2} than non-TT cultivars but with similar seed weight. The results indicated that the yield penalty of the TT trait is associated with the development of a smaller source and sink capacity. Harvest index for TT cultivar was shown to vary from 0.09 to 0.45 in multi-environment studies by Robertson *et al.* (2002b). In our study, the HI of TT cultivars (0.27 and 0.34) was similar to the non-TT (0.26 and 0.34) cultivars during 2011 and 2012.

In single cultivar comparisons of hybrid and OP canola from a multi-location experiment in Canada, Mahli *et al.* (2007) found that hybrid cultivars produced more biomass than OP cultivars and this was reflected in their higher seed yield. In this study, hybrid canola also produced more biomass than OP cultivars but with lower HI, which resulted in similar seed yields in 2011. However in the drier year 2012, hybrid canola produced 34% higher biomass and 28% higher seed yield (P=0.007) than the OP cultivars. These results suggest that in environments with high winter rainfall, hybrids are a better option over OP cultivars due to their higher biomass and seed yield production.

RUE values of canola (TT, non-TT, hybrid, OP) were close to those reported by Robertson *et al.* (2002b) for TT and non-TT cultivars. In our study, RUE of mustard was close to the values reported in a low rainfall area by O'Connell et al. (2004) but lower than widely reported values of 2.02-3.58 g MJ^{-1} (Mendham *et al.* 1981; Mendham *et al.* 1990; Morrison and Stewart 1995), which were derived from experiments in high rainfall areas. O'Connell *et al.* (2004) showed that RUE was not sensitive to water stress and it is site specific whereas our study in two contrasting years showed that RUE of canola and mustard was affected by the environmental stresses.

3.5.2. Responsiveness to Nitrogen

Canola and mustard responded similarly to N application in both years. On average, N improved total dry matter by 18% and 23% in 2011 and 2012 respectively, and improved seed yield by 38% in 2011 and 30% in 2012. Overall improvement of HI with N was less in 2012 than 2011. These differences between the seasons may be due to water stress during flowering and pod-development due to less than average rainfall during July, August and September in 2012 which can affect N uptake and utilisation (Sadras 2004; Sinclair and Rufty 2012). Diepenbrock (2000) considered flowering as the most critical stage to influence seed yield even under favourable conditions, due to decrease in the total leaf area by shading, firstly by onset of flowering and then by pods (Gabrielle *et al.* 1998). Richards and Thurling (1978) also found significant reduction in dry matter and yield components with water stress at flowering and pod development.

Several studies have reported pod number to be the main factor responsible for seed yield in canola (Scott *et al.* 1973; Beversdorf *et al.* 1988; Wright *et al.* 1988;

Hocking *et al.* 1997d). Pods per plant and pods m^{-2} can be affected greatly by developmental and environmental factors as well as with water and nutrients (Allen and Morgan 1972; Tayo and Morgan 1975; Diepenbrock 2000) - this was also the case in this study as in 2012 pods m^{-2} and seed yield were about half that in 2011. Seeds m^{-2} was the most influential yield component in this study, which was improved by N. Even though the seasonal differences in gain yield seems to be due to the differences in pod m^{-2}, seed yield was more strongly correlated with seeds m^{-2} than pods m^{-2} ($r = 0.99$ and $r = 0.97$ for canola and $r = 0.50$ and $r = 0.61$ for mustard in 2011 and 2012 respectively). The number of seeds m^{-2} was strongly correlated with shoot dry matter at rosette stage and the correlation improved through the growing season for canola. In mustard seeds m^{-2} was strongly correlated with pod m^{-2} but seed yield was correlated with dry matter at flowering and at maturity.

Canola and mustard were more responsive to an early N application at rosette than later at green-bud or at flowering. When N was applied at rosette stage, the total dry matter at maturity was 26% and 28% higher than the control in 2011 and 2012 respectively, whereas improvement in dry matter was only 13% and 9% for the application of N at green-bud and <1% and 12% for N applied at flowering over control in 2011 and 2012, respectively. While responses to applications of N after green bud were lower, the responsiveness of canola and mustard to N late in development meant that the highest total dry matter and seed yield was observed in the non-limiting N treatment in both years. Canola and mustard achieved 85% and 94% of the yield by non-limiting N treatments when N was applied at rosette stage in 2011 and 2012 respectively. Further delay in N application after the rosette stage resulted in a larger yield penalty. The importance of the period from rosette to green-bud stage for N application was identified in previous studies by assessing plant N status at various growth stages

(Bernardi and Banks 1993; Hocking *et al.* 1997b). In this study, delay in N after rosette stage caused a small improvement in HI in 2011 but it was associated with a yield penalty. In 2012, Grain yield achieved by applying 100 kg N ha^{-1} at rosette stage was comparable with the split application of same amount on five key growth stages which the inability of crop to early uptake the N under water limited conditions as volatilization losses in winter and early spring are generally low in these environments.

As discussed earlier, shoot dry matter and seed m^{-2} were the main components for attaining higher yield of canola and mustard and both were improved with addition of N at the rosette stage. Seed yield of mustard appears to rely more heavily on post-flowering growth than canola in which yield was more strongly influenced by vegetative growth up to flowering. Development pattern of mustard cultivars used in this study would make them more sensitive to water stress during post-flowering period than canola. HI content remained relatively stable across the N treatments. Independence of seed yield from HI in canola mustard observed in this study was also reported for different *brassica* species by Lewis and Thurling (1994).

3.6. Conclusions

Seed yield of canola and mustard in this Mediterranean environment was mainly determined by shoot dry matter rather than changes in harvest index. Therefore, early vigour and early dry matter production is necessary for achieving higher seed yields in canola. Surprisingly, mustard seed yields were lower than canola in a season with a dry finish, which appears to be related to a strong relationship between post-flowering growth and seed yield in mustard. Even though triazine tolerant cultivars showed DM and yield penalty relative to the non-TT canola, their inclusion in cropping programs may be justifiable due to weed control benefits. Hybrid canola cultivars need more dry matter produce per unit seed yield (i.e. lower HI) than the open pollinated cultivars of canola so they appear less efficient but still be an option for areas with high winter rainfall.

Nitrogen improved seed yield of canola and mustard by increasing dry matter production and yield components. Nitrogen at rosette stage (GS30) is critical for achieving higher yields in these environments. Application of N at the rosette stage was shown to promote inflorescence development and improve sink capacity, which was ultimately reflected in greater seed m^{-2}, and higher seed yield.

3.7. Acknowledgements

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3.8. Supplementary Tables

Table S1: Effect of cultivars and N on pods m^{-2} (10^{3}) for 2011 and 2012

Year	Rate	Targeted GS for N	AV Garnet	FighterTT	Hyola 555TT	Hyola 575CL	Oasis	Varnua
2011	0		6.2	6.2	4.8	6.7	5.9	7.8
	100	30,63, 71	6.7	5.6	6.0	6.7	9.1	8.2
	100	51,67	8.0	8.2	5.9	6.3	12.7	8.2
	100	63,71	6.9	8.5	6.7	7.8	12.4	7.3
	200	30,51,63,67,71	9.9	7.5	7.2	8.0	15.3	9.8
	lsd		3.3					
2012	0		2.6	2.6	2.9	2.8	3.4	1.6
	100	30	3.4	3.7	3.4	3.1	5.9	2.3
	100	51	3.4	4.6	4.5	3.9	3.5	2.5
	100	63	3.8	4.0	2.6	4.4	3.8	2.2
	100	30,51,63,67,71	3.2	4.1	3.3	3.5	4.1	2.5
	200	30,51,63,67,71	3.0	4.7	4.0	3.6	5.6	2.5
	lsd		1.2					

Table S2: Correlation coefficients between seed yield, yield components, dry matter at various growth stages and HI including all N treatments for canola cultivars grown at Roseworthy during 2011. (n= 20)

	Dry matter					Yield components					
Canola 2011	Ro	GB	Fl	PD	Ma	Pods m^{-2}	Seeds m^{-2}	Seed Pod^{-1}	Seed wt.	HI	GY
DM Ro	-										
DM GB	0.71***	-									
DM F	0.69***	0.92***	-								
DM PD	0.68***	0.91***	0.98***	-							
DM M	0.77***	0.90***	0.95***	0.97***	-						
Pods m^{-2}	0.28	0.40	0.48*	0.42	0.44	-					
Seeds m^{-2}	0.53*	0.78***	0.90***	0.91***	0.88***	0.52	-				
Seed Pod^{-1}	-0.33	-0.34	-0.41	-0.39	-0.39	-0.67**	-0.25	-			
Seed weight	0.28	0.26	0.13	0.14	0.18	0.16	-0.11	-0.58**	-		
HI	-0.56**	-0.32	-0.23	-0.26	-0.35	0.16	0.09	0.27	-0.37	-	
Seed Yield	0.58**	0.83***	0.93***	0.94***	0.92***	0.54*	0.99***	-0.33	0.03	0.02	-

Table S3: Correlation coefficients between seed yield, yield components, dry matter at various growth stages and HI including all N treatments for canola cultivars grown at Roseworthy during 2012.(n= 24)

	Dry matter					Yield components					
Canola	Ro	GB	Fl	PD	Ma	Pods m^{-2}	Seeds m^{-2}	Seed Pod^{-1}	Seed wt.	HI	GY
DM Ro	-										
DM GB	0.29	-									
DM F	0.28	0.54**	-								
DM PD	0.04	0.15	0.03	-							
DM M	0.56**	0.70***	0.4	-0.1	-						
Pods m^{-2}	0.05	-0.06	-0.01	-0.07	0.06	-					
Seeds m^{-2}	0.54**	0.73***	0.43*	0.00	0.92***	-0.03	-				
Seed Pod^{-1}	0.28	0.38	0.38	0.14	0.45*	0.18	0.44*	-			
Seed weight	-0.44*	-0.53**	-0.51*	0.11	-0.63**	0.07	-0.77***	-0.41*	-		
HI	-0.29	-0.16	-0.23	0.27	-0.51*	-0.10	-0.19	-0.34	0.25	-	
Seed Yield	0.54**	0.76***	0.38	0.04	0.94***	0.01	0.97***	0.38	-0.62**	-0.18	-

Table S4: Correlation coefficients between seed yield, yield components, dry matter at various growth stages and HI including all N treatments for mustard cultivars grown at Roseworthy during 2011.(n= 10)

	Dry matter					Yield components					
Mustard	Ro	GB	Fl	PD	Ma	Pods m^{-2}	Seeds m^{-2}	Seed Pod^{-1}	Seed wt.	HI	GY
DM Ro	-										
DM GB	0.68*	-									
DM F	0.50	0.63	-								
DM PD	0.59	0.68*	0.97***	-							
DM M	0.57	0.69*	0.98***	0.99***	-						
Pods m^{-2}	-0.54	-0.30	0.07	-0.09	-0.06	-					
Seeds m^{-2}	-0.62	-0.29	0.20	0.02	0.07	0.83**	-				
Seed Pod^{-1}	0.63	0.35	0.27	0.35	0.37	-0.75*	-0.49	-			
Seed weight	0.86**	0.67*	0.61	0.75*	0.70*	-0.51	-0.61	0.51	-		
HI	-0.41	-0.39	0.05	-0.10	-0.09	0.82**	0.76*	-0.41	-0.46	-	
Seed Yield	0.27	0.37	0.89***	0.82**	0.84**	0.41	0.50	0.08	0.37	0.45	-

Table S5: Correlation coefficients between seed yield, yield components, dry matter at various growth stages and HI including all N treatments for mustard cultivars grown at Roseworthy during 2012.(n= 12)

	Dry matter					Yield components					
Mustard	Ro	GB	Fl	PD	Ma	Pods m^{-2}	Seeds m^{-2}	Seed Pod^{-1}	Seed wt.	HI	GY
DM Ro	-										
DM GB	0.67*	-									
DM F	0.46	0.53	-								
DM PD	0.57	0.52	0.23	-							
DM M	0.38	0.46	0.88***	0.17	-						
Pods m^{-2}	-0.77**	-0.66*	-0.32	-0.46	-0.18	-					
Seeds m^{-2}	-0.67*	-0.41	-0.03	-0.25	0.10	0.83***	-				
Seed Pod^{-1}	0.44	0.62*	0.02	0.17	-0.07	-0.55	-0.59*	-			
Seed weight	0.82**	0.66*	0.58*	0.34	0.55	-0.76**	-0.73**	0.56	-		
HI	-0.78**	-0.62*	-0.54	-0.40	-0.58*	0.66*	0.63*	-0.26	-0.83***	-	
Seed Yield	-0.06	0.17	0.63*	0.00	0.79**	0.30	0.61*	-0.26	0.07	0.0	-

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Chapter 4

4. Nitrogen and water use efficiency of canola and mustard with nitrogen applications at key phenological stages.

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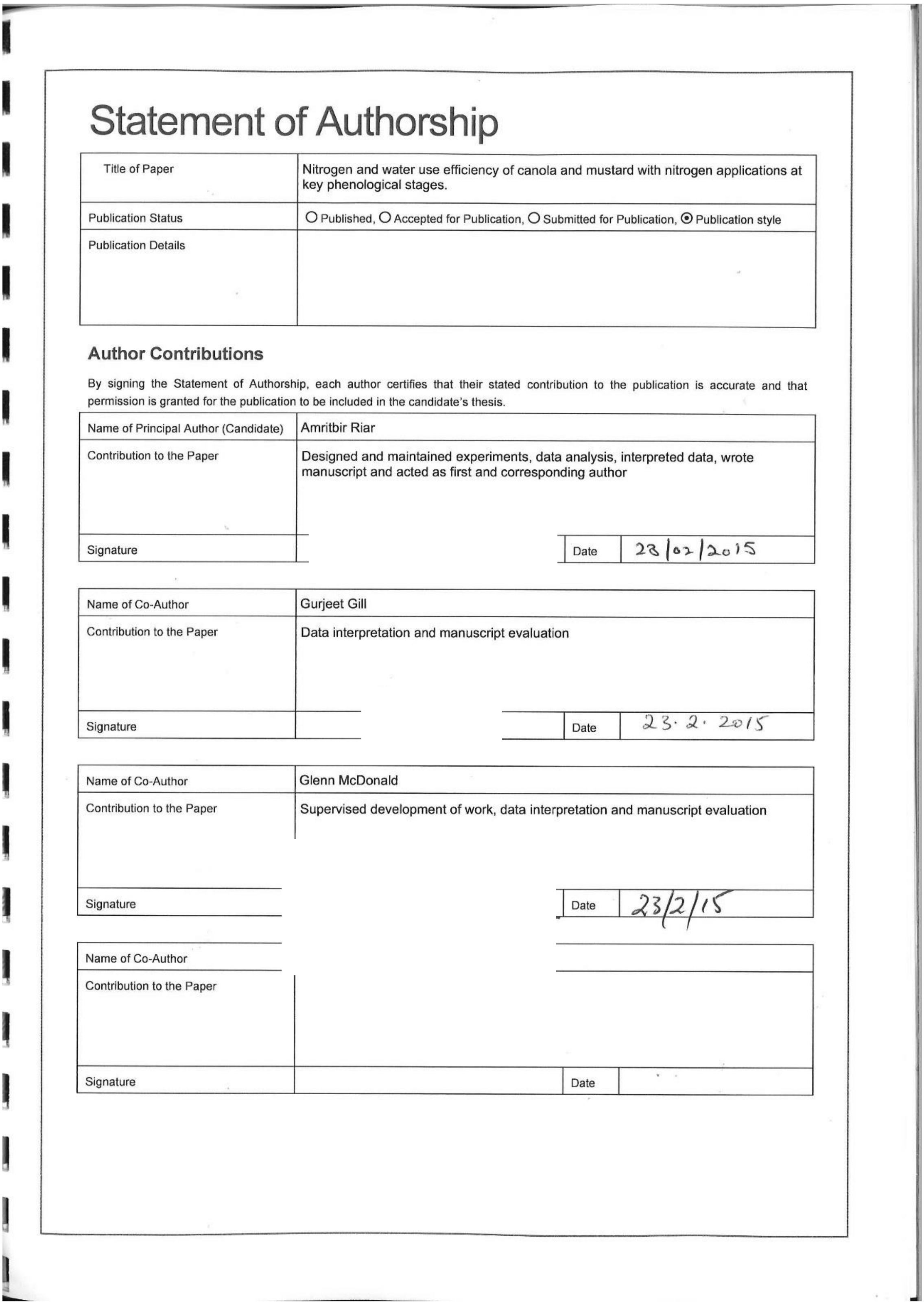

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Nitrogen and water use efficiency of canola and mustard with nitrogen applications at key phenological stages.

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4.1. Abstract

Plant breeding and agronomic management have contributed to the improvement in canola and mustard yields in Australia. Canola and mustard have high demands for N but water and N use efficiency is often low. Poor use of soil water and N may be partly responsible for the sub-optimal performance of canola. Strategic use of N may improve efficiency but there is limited information on the influence of N availability at key growth stages on N accumulation, water use and water use efficiency. In this study we hypothesise that the timing and rate of post-sowing N application at targeted phenological growth stages can improve N and water use and their efficiencies in canola and mustard. Field experiments were conducted in South Australia during the 2011 and 2012, growing seasons with contrasting water availabilities. Two mustard and four canola cultivars including two triazine tolerant (TT) and two non-TT cultivars were evaluated under different N application strategies comprising three N rates (0, 100 and 200 kg N/ha as granular urea) and different timings of application. Seasonal water availability had a large influence on crop water use, N uptake, N use efficiency (NUE), agronomic N efficiency and apparent recovery of N. In the higher rainfall season (2011), mustard used more water than canola but canola and mustard used similar amounts of water in the drier season (2012). Nitrogen rate and timing did not influence the total water use of canola and mustard but influenced the partitioning of pre- and post-flowering water use. Water extraction depth at flowering was increased with N rate but all cultivars extracted water

from a similar depth of soil at maturity. Among canola cultivars, TT and non-TT cultivars used a similar amount of water but TT cultivars had lower N uptake and NUE for seed yield (NUE_{SY}) possibly due to their lower radiation and transpiration use efficiency. Low NUE_{SY} in these environments was mainly related to limitation of N uptake and low N uptake efficiency rather than physiological N efficiency. Nitrogen increased the water use efficiency (WUE) of canola and mustard cultivars but a trade-off between WUE and NUE was found as indicated by a strong negative correlation between them.

4.2. Introduction

Plant breeding has improved adaptation and productivity of canola and mustard in Australia. Canola and mustard have higher demands for N but lower water and N use efficiency as compared to cereals. Inefficient use of water and N may be partly responsible for sub-optimal performance of canola. To achieve the full yield potential of canola and mustard, it is necessary to overcome constraints limiting their growth and production in such environments. It will be difficult to realise the full benefits of genetic improvement without improving the N and water use of these crops (Sinclair and Rufty 2012). Understanding the role of N availability at various growth stages in improving N and water use of canola and mustard could play an important role in enhancing productivity in water limited environments with low soil fertility.

Nitrogen is an expensive and difficult to manage input in environments with variable rainfall, which is a distinguishing feature of Mediterranean environments. Nitrogen losses from agricultural systems are also becoming a serious concern. Recovery of N in crops is generally less than 50% (Fageria and Baligar 2005), which cannot be justified from environmental and economic perspectives (Grant *et al.* 2002b). Campbell *et al.* (2004) stated that N yield is a function of plant available water as water is a major driver of grain yield in rainfed systems. Moreover water deficits at a critical growth stage can limit N uptake and utilisation in plants (Benjamin *et al.* 1997) and can reduce crop response to N fertilisers. Many studies have shown the importance of the effects of water availability on N response of crops and *vice-versa* (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012). According to Sadras (2004), the gap between actual and potential yield in a rainfed system was lower when water and N equally co-limited the growth of a wheat crop.

In rainfed environments, water is a limiting resource and its availability depends on the amount of water stored during the fallow and the amount of growing season rainfall. It is widely accepted that the management of fertiliser inputs is one of the most important tools for the improvement of WUE in these environments (Cooper *et al.* 1987; Angus and Van Herwaarden 2001; Sadras and Roget 2004). Previous studies on the water use of oilseed crops (Johnston *et al.* 2002; Campbell *et al.* 2007; Angadi *et al.* 2008; Gan *et al.* 2009) have not investigated the influence of N on water uptake from different depths in the soil profile. The effects of N applications on targeted growth stages on growth and seed yield of canola and mustard were earlier reported (Riar *et. al.* Unpublished). In the study reported here, we tested the hypothesis that the timing and rate of post-sowing N application targeted at different phenological growth stages can influence NUE and WUE in canola and mustard.

4.3. Materials and Methods

4.3.1. Site description

Field experiments were conducted at the Roseworthy farm of the University of Adelaide (latitude 34.53 °S; longitude 138.72 °E), South Australia during the 2011 and 2012 growing seasons. The long term annual average rainfall for Roseworthy is 440mm with a growing season average rainfall (defined in South Australia as rainfall from April to October (French and Schultz 1984) of 329 mm.

The main soil type of the experimental sites was Chromosol with an alkaline trend down the profile (Isbell 2002). In order to estimate soil moisture and nitrate-N up to a depth of 100 cm, nine soil cores were taken two days prior to sowing in both seasons by using a 4 cm hydraulic core. Soil samples were taken at five depths from across the sites at sowing, bulked, dried at 40C and sieved (<2mm) for analysis by a commercial laboratory (CSBP Perth, Western Australia). The amount of the mineral-N (ammonium + nitrate) in the 0-100 cm layer was 77 kg ha^{-1} and 71 kg ha^{-1} in 2011 and 2012, respectively. Detailed soil characteristics of the experimental sites are given in Table: 1.

4.3.2. Experimental design and crop management

Detailed information on the experimental design and crop management practices used in this study have been reported in chapter: 3. In brief, two mustard (Varuna and Oasis) and four canola cultivars, including two triazine-tolerant (TT - Fighter TT and Hyola555TT) and two non-TT cultivars (AV Garnet and Hyola575cl), were evaluated under different N application strategies, comprising three N rates (0, 100 and 200 kg N ha^{-1} as granular urea; 46% N) and different timings of application (Table: 2). In 2011 the treatments were arranged in a split-split plot design with time of sowing as the main-plots, canola and mustard cultivar as the sub-plots and N treatments as sub-sub-plots in three replications. In 2012, only one time of sowing was used so the treatments were arranged in a split plot design with cultivars in main-plots and N treatments in sub-plots with three replications. The trials were sown with a cone seeder with knife point drill and press wheels at a depth of 25mm. Plots were 10 metres long consisting of six rows with a 250 mm inter-row width. Basal fertilisers were 10 kg N ha^{-1} and 11 kg P ha^{-1} as diammonium phosphate (DAP) applied at sowing and 100 kg S ha^{-1} as a pre-planting gypsum application. Seeding rates were adjusted for each cultivar based on their seed weight and germination test to achieve a plant establishment of 35 plants m^{-2}. Plant numbers were counted after crop establishment and it showed that on average there was 84% and 82 % establishment in 2011 and 2012, respectively. Weeds and diseases were managed with standard agronomic practices and overall weed and disease incidence was minimal during both years.

Table 1: Soil characteristics of each site used during 2011 and 2012. Analyses were conducted by CSBP Soil and Plant Analysis Laboratories, Perth WA using the methods described in Rayment and Lyons (2011).

Year	Layer (cm)	Ammonium N[11] (mg kg^{-1})	Nitrate N[12] (mg kg^{-1})	Colwell P[13] (mg kg^{-1})	Colwell K[14] (mg kg^{-1})	Sulphur[15] (mg kg^{-1})	Organic C[16] (%)	Conductivity[17] (dS/m)	pH level H2O (pH)[18]	Boron[19] (mg kg^{-1})	Calcium carbonate[20] (%)
2011	0-20	7.0	4.0	23.0	580	7.2	0.99	0.211	7.5	2.07	9.14
	20-40	3.0	2.0	7.0	220	7.2	0.57	0.170	8.2	2.39	30.40
	40-60	2.0	1.0	10.0	222	18.0	0.32	0.230	8.1	6.18	47.84
	60-80	2.0	2.0	3.0	424	56.8	0.20	0.464	8.5	17.03	40.80
	80-100	1.0	5.0	<2.0	543	66.7	0.15	0.404	8.7	25.50	37.44
2012	0-20	12.7	10	56.7	509	20.1	1.21	0.300	7.7	1.89	0.98
	20-40	5.3	4.0	13.7	192	12.5	0.66	0.200	8.6	3.25	20.39
	40-60	4.0	1.6	9.7	132	16.8	0.34	0.210	8.8	4.21	45.76
	60-80	3.0	1.7	5.0	179	29.6	0.25	0.428	9.1	9.62	53.53
	80-100	2.3	2.0	3.0	276	55.0	0.19	0.622	9.3	13.19	52.49

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[11] Method No. 7C2b pp 130
[12] Method No. 7C2b pp 130
[13] Method No. 9B &18A1 pp 162 & 385
[14] Method No. 9B &18A1 pp 162 & 385
[15] Method No. 10D1 pp 223
[16] Method No. 6A1 pp 68
[17] Method No. 4A1, 4B3 & 3A1 pp 38 & 20
[18] Method No. 4A1, 4B3 & 3A1 pp 38 & 20
[19] Method No. 12C1 & 12D1 pp 244
[20] Method No. 19B2 pp 420

Nitrogen treatments were designed to generate a range of crop biomass and canopy size (Table: 2) and targeted at specific growth stages. A control treatment with no N (N0) and a high N control (N200) in which a total of 200kgN ha^{-1} was applied in five equal split applications (rosette (GS30), green-bud (GS51), start of flowering (GS61), start of pod filling (GS67) and 10% pod maturity (GS71) was used to maintain a steady supply of N throughout the season. The growth stages were recorded by using the BBCH canola scale (Lancashire *et al.* 1991). These controls were designed to provide a boundary function of crop response to N in both years. All other treatments were designed to examine the effects of N supply at a specific growth stage on water and N use and their efficiencies. Nitrogen was spread by hand at the desired growth stage either when the soil was wet or if rainfall was forecast within 24h after application. On an average, there was 13.2mm and 9.92mm rainfall in a week time after N applications in 2011 and 2012 respectively.

Table 2: Details of N rate, number of split applications and growth stages for N application used during 2011 and 2012

Year	Rate	Splits	Targeted Growth Stages (BBCH scale)
2011+2012	0	0	0
2011	100	3	30,63,71
2011	100	2	51,67
2011	100	2	63,71
2012	100	1	30
2012	100	1	51
2012	100	1	63
2012	100	5	30,51,63,67,71
2011+2012	200	5	30,51,63,67,71

4.3.3. Measurements and sampling

Soil moisture content for 0-100 cm depth was measured pre-sowing, at flowering and at maturity using a 4cm hydraulic core in 2011 and 2012. Cores were sub-divided into five layers each of 20cm depth to assess the differences in water extraction at various depths. In addition, two contrasting cultivars were selected (based upon the differences in growth and seed yield in 2011) to frequently (5-10 day intervals) monitor changes in soil water in the profile down to one metre with the frequency domain reflectometry (capacitance) method using a Diviner 2000 (Charlesworth 2000). The change is soil water over 0-100 cm was used to estimate crop water use (CWU) assuming no drainage below the root zone:

CWU = Growing season rainfall - (Soil moisture at harvest – soil water at sowing).

Water use efficiency (WUE) was calculated as seed yield divided by water used from sowing to harvest. To examine variation in transpiration efficiency (TE) due to genetic variation, in 2011 the abundance of the stable isotopes ^{12}C and ^{13}C relative to PeeDee Belemnite (PDB) was measured using mass spectrometry (elemental analyser, E A1108, Series1: Carlo Erba Istrumentazione, Milan, Italy). Measurements were based on leaves collected from four random plants per plot at the rosette stage in the nil N control plots. The leaf material was dried at 80 °C for 48 hours, ground twice and the second time in a ball mill before the isotopic composition was measured. Carbon isotope composition values ($\delta\,^{13}C$) were converted to Δ by assuming isotopic composition of air to -8 ‰.

To measure the N content of shoots, four random plants were taken from each plot at 50% flowering (GS65) and maturity (GS99), and dried in an oven at 80 °C for 48 hours. Nitrogen content was determined with a LECO combustion analyser, where plant samples were loaded into the combustion tube (at 950˚C) and flushed with oxygen. The efficiency of N for canola and mustard was calculated by using the following formulae (Fageria and Baligar 2005; Rathke *et al.* 2006):

$$\text{N use efficiency for seed yield (NUE}_{SY}\text{) (kg kg}^{-1}\text{)} = \frac{\text{Seed yield}}{\text{N supply (Fertiliser+Soil N)}}$$

$$\text{N-uptake efficiency (kg kg}^{-1}\text{)} = \frac{\text{Total N uptake}}{\text{N supply}}$$

$$\text{Agronomic efficiency (kg kg}^{-1}\text{)} = \frac{G_F - G_c}{F}$$

$$\text{Apparent recovery (\%)} = \frac{N_F - N_c}{F} \times 100$$

$$\text{Physiological efficiency (kg kg}^{-1}\text{)} = \frac{Y_F - Y_c}{N_F - N_c}$$

where G_F and G_C are the seed yield of the fertilised and unfertilised plots, Y_F and Y_C are the biological yield of the fertilised and unfertilised plots, N_F and N_C is the N contained in biological yield (kg ha^{-1}) of fertilised and unfertilised plots, and F was the amount of fertiliser N applied as granular urea (McDonald 1989; Fageria and Baligar 2005). Agronomic efficiency reflects the efficiency with which applied N is used and physiological efficiency can be viewed as the response of crop to additional N uptake from fertiliser. The total soil N measure at the start of the seasons was used to estimate N supply instead of seasonal N mineralisation.

4.3.4. Statistics

The data obtained from the experiment were analysed by analysis of variance (ANOVA) using the GenStat statistical analysis software (15th edition). Orthogonal comparisons were used to compare the different groups of cultivars and N treatment based on single degree of freedom comparisons (VSN 2013). For other statistical comparisons, the least significant difference (LSD) at 5% level of probability (P=0.05) was used to compare the treatments. In some cases where the small degrees of freedom limited the statistical power of the analysis, LSD at 10% level of probability was also used (Nuzzo 2014).

4.4. Results

4.4.1. Water use pattern and efficiency

4.4.1.1. Pre-flowering and post –flowering water use

Growing season (April-October) rainfall was similar in both years (232 mm in 2011 and 220 mm in 2012) but the annual rainfall (January-December) was 102 mm lower in 2012 (292 mm) than 2011 (394 mm). In 2011, the season had a wet start with above-average rainfall in February and March so the soil water to one metre depth at sowing was 220mm whereas 2012 had a dry start with starting soil water of 70 mm. In 2012, rainfall during the spring (September-November) was less than half of that received over this period in 2011 (Table: 3). From here on, we consider 2011 as a wet year (452 mm soil water during crop growing period) and 2012 as a drier than average year.

Table 3: Rainfall and water availabilities at various phases of two growing years

	Annual rainfall	GS rainfall	Pre-sowing rainfall (Jan-April)	Pre-flowering rainfall (May-August)	Post-flowering rainfall (Sept-Nov)	Soil water at sowing
2011	394	232	140	132	94	220
2012	292	220	74	161	45	70
Long term average	440	329	98	202	118	

Total water use by canola and mustard cultivars differed between the two years. On average, total water use by canola and mustard cultivars was 349mm during 2011 and 171mm during 2012 (Table: 4). The total water use by mustard was 2.5% greater than canola in 2011 (P= 0.089) but both crop species used similar amount of water in 2012. Mustard used less water than canola during the pre-flowering period (3.7% in 2011: P=0.094; 29% in 2012: P <.001) but during the post-flowering period mustard used more water than canola in both years (14% in 2011: P=0.015; 26% in 2012: P= 0.005).

In 2011, N treatments affected the partitioning of water use between pre-flowering and post-flowering growth periods without affecting the total water use of canola and mustard (Table: 4). Nitrogen treatment with a total 200 kg N ha^{-1} split between five key growth stages used 22 mm and 33 mm more water in the pre-flowering phase than 100 kg N ha^{-1} in three splits and the control, respectively. Consequently, the crop with 200 kg N ha^{-1} used 16 and 26 mm less water than 100 kg N ha^{-1} in three splits and control during the post-flowering phase (Table: 4).

Table 4: Total, Pre and post flowering water use and Water use efficiency of different cultivars under N rates of 0 kg N ha^{-1} (N0), 100 kg N ha^{-1} (N100) and 200 kg N ha^{-1} (N200) during the growing season of 2011 and 2012.

		Water use			WUE	
Cultivars	N treatments	Pre-Flowering	Post-Flowering	Total	GY	DM
2011						
AV-Garnet	N0	244.4	106.3	350.7	5.5	19.9
	N100	247.4	80.2	327.6	7.6	27.7
	N200	277.7	68.7	346.4	8.3	29.8
Fighter TT	N0	239.7	107.3	347.0	3.7	14.3
	N100	249.5	110.8	360.3	4.5	16.6
	N200	275.1	78.6	353.7	4.5	15.6
Hyola555TT	N0	231.7	113.8	345.5	2.8	11.3
	N100	234.6	119.2	353.8	4.9	17.8
	N200	257.7	81.9	339.6	5.3	17.6
Hyola575CL	N0	223.4	127.1	350.5	4.7	23.7
	N100	246.1	77.8	323.9	7.0	30.2
	N200	269.1	85.8	354.9	8.4	33.0
Oasis	N0	222.7	112.6	343.7	3.9	17.9
	N100	232.4	127.3	359.7	5.2	24.7
	N200	259.0	98.9	357.9	7.4	25.2
Varuna	N0	230.9	117.9	348.8	4.8	25.6
	N100	248.0	109.4	357.4	6.6	29.4
	N200	250.1	114.2	364.3	6.6	27.1
	lsd	29.1	41.4	31.0	1.9	8.2
2012						
AV-Garnet	N0	83.2	105.2	188.4	5.0	14.0
	N100	112.7	62.6	175.3	7.3	18.4
	N200	119.0	66.5	185.5	6.3	18.3
Fighter TT	N0	108.3	46.0	154.3	3.9	10.0
	N100	108.0	69.6	177.7	4.8	14.4
	N200	95.3	96.8	192.0	5.5	20.1
Hyola555TT	N0	117.5	53.1	170.7	5.6	19.1
	N100	111.1	64.0	175.1	5.9	16.8
	N200	124.4	51.2	175.6	6.7	21.4
Hyola575CL	N0	106.9	43.0	149.8	7.3	25.1
	N100	124.1	49.5	173.7	8.0	25.1
	N200	121.9	52.8	174.6	8.4	25.5
Oasis	N0	61.8	90.6	146.3	3.7	10.8
	N100	81.8	97.4	179.1	5.8	18.6
	N200	61.2	98.0	159.2	6.6	24.7
Varuna	N0	93.7	64.2	157.9	3.2	17.1
	N100	89.0	89.6	178.6	5.0	20.8
	N200	85.9	78.6	164.5	5.9	29.4
	lsd	16.0	28.0	27.3	2.7	9.4

In 2012, pattern of pre-flowering and post-flowering water use was similar to 2011 in canola and mustard cultivars (Table: 4). However, there was a significant Cultivar ×Nitrogen interaction for total crop water use. Total water use over the growing season by canola with 100 kg N ha^{-1} at the rosette stage was similar to 200 kg N ha^{-1} in five splits and the control. However, mustard used 17mm more water with 100 kg N ha^{-1} applied at the rosette stage than 200 kg N ha^{-1} applied as five splits (Table: 4). In all canola and mustard cultivars, a single application of 100 kg N ha^{-1} used more water in pre-flowering growth period than the same N dose in three splits and the control. All N treatments used similar amounts of soil water during the post-flowering period.

Cumulative water use of two canola cultivars with contrasting early vigour (AV Garnet and FighterTT) was measured with a capacitance probe at 16 different times during the growing season. This revealed that water use of these two cultivars was not significantly different at any sampling time. In the pre-flowering period, crop water use in treatments supplied with N was higher than the control but in the post-flowering period all treatments used the same amount of water (Fig: 1). Total water used by the crop was similar between N treatments and the control. Water was extracted from the soil profile to 90 cm but some soil water accumulated during the growing season below 70 cm (Fig: 1).

4.4.1.2. Water Use Efficiency (WUE)

Carbon isotope discrimination revealed that there were no significant genetic differences in stable carbon ratios ($^{13}C/^{12}C$) among the cultivars (Δ range from 18.74 ‰ to 19.79 ‰). Orthogonal contrasts suggested the mean water use efficiency for seed yield (WUE_{GY}) of canola and mustard was very similar in 2011 but the mean WUE_{GY} of canola was 1.2 kg ha^{-1} mm^{-1} higher than mustard in 2012 (P=0.085) (Table: 4). However in both years

TT cultivars of canola had significantly lower WUE_{GY} than non-TT cultivars with Fighter TT having the lowest WUE in both seasons.

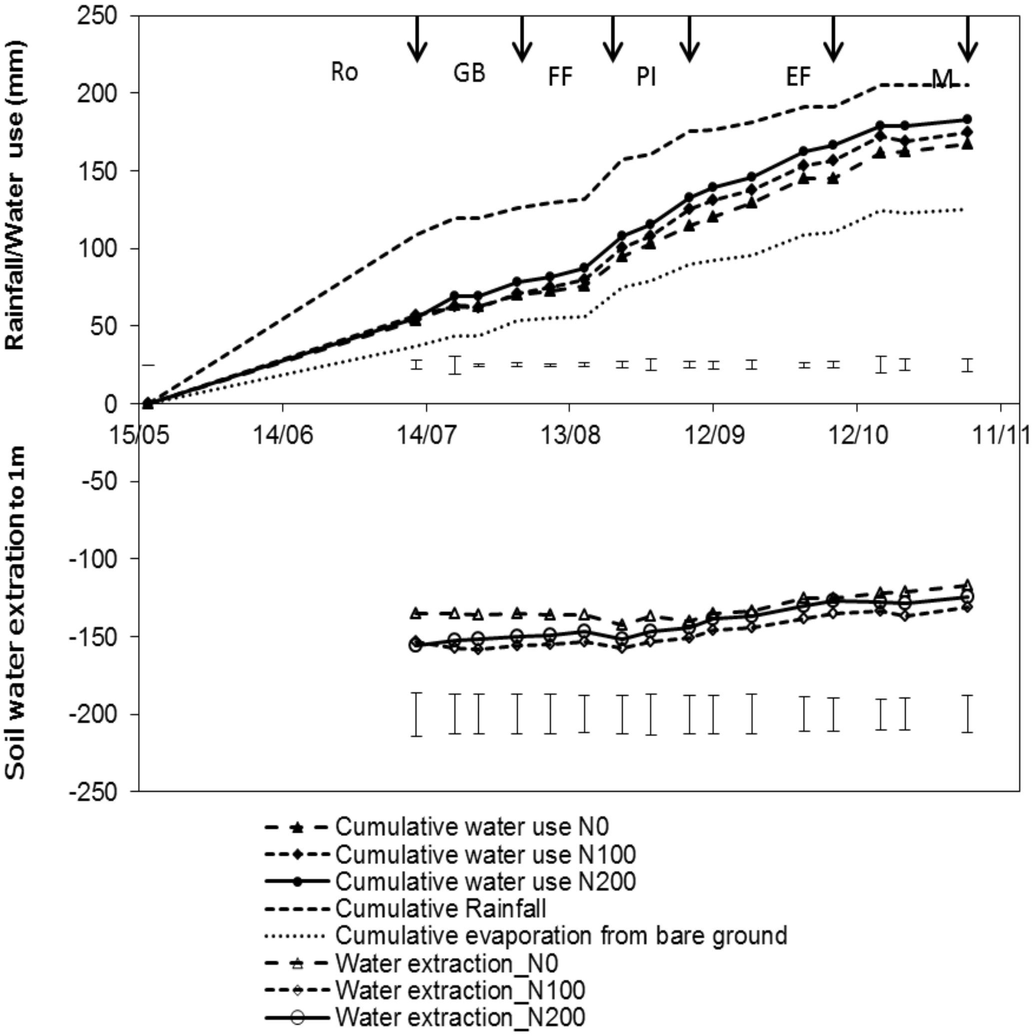

Figure 1: Water use and water extraction patterns of canola cultivars under different N regimes during the growing season of 2012. Values are the mean of AV Garnet and Fighter TT cultivars as no significant difference between varieities in water use and soil water extraction was observed at any time.where vertical bars represent the LSD for each point of measurement.

Water use efficiency of total dry matter (WUE_{DM}) was similar for canola and mustard in both years. In wet year, non-TT cultivars had 11.9 kg ha^{-1} mm^{-1} higher than WUE_{DM} than TT cultivars but non-TT and TT cultivars had similar WUE_{DM} during dry year. Open-pollinated and hybrid cultivars had similar WUE_{DM} in wet year but Open-pollinated cultivars had lower WUE_{DM} than hybrid cultivars by 6.3 kg ha^{-1} mm^{-1}.

Applying N improved WUE_{GY} and WUE_{DM} in both years. In 2011, the highest WUE_{GY} was achieved with 200 kg N ha^{-1} in five splits followed by 100 kg N ha^{-1} in three splits and control (Table: 4). In 2012, WUE_{GY} did not differ between 200 kg N ha^{-1} and the single application of 100 kg N ha^{-1} at the rosette stage but both were higher than the WUE_{GY} in the control (Table: 4). Trends for WUE_{DM} were in reverse order than WUE_{GY}. In 2011, WUE_{DM} did not differ between 200 kg N ha^{-1} and the single application of 100 kg N ha^{-1} at the rosette stage but both were higher than the WUE_{GY} in the control. In 2012, the highest WUE_{GY} was achieved with 200 kg N ha^{-1} in five splits followed by 100 kg N ha^{-1} in three splits and control (Table: 4).

4.4.1.3. Water distribution in the soil profile

As a result of good pre-sowing rainfall in 2011, there was >4 cm soil water in all 20cm depth increments at the time of sowing. Non-TT canola showed deeper extraction of moisture with increasing rate of N at mid-flowering. For example, crops grown with 100 kg N ha^{-1} extracted soil water up to 70 cm depth whereas those grown with 200 kg N ha^{-1} extracted soil water up to 90 cm depth (Fig: 2 a,b,c). TT canola appeared to extract less soil water than non-TT canola at the mid-flowering stage. The depth of soil water extraction at flowering was also less in TT than non-TT canola. For example with 200 kg N ha^{-1}, TT canola extracted soil water up to 50 cm compared to 90 cm in non-TT canola (Fig: 2 c,f). In 2011, the soil profile under mustard at mid-flowering was wetter than non-TT and TT canola. However, there was no difference between the crop types and N management on soil water content down the profile at maturity. Therefore, the amount of water used by different crop types from mid-flowering to maturity was quite different.

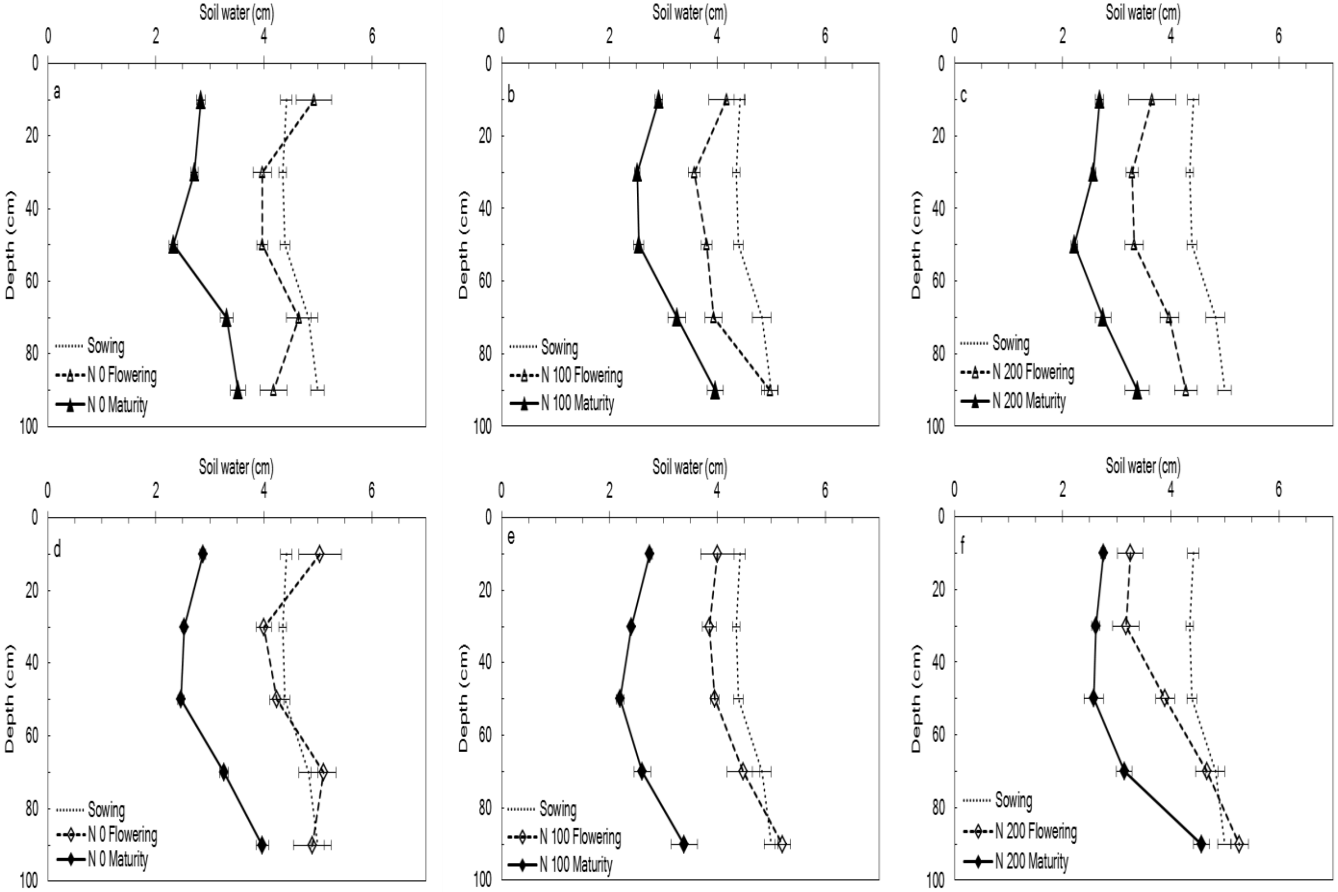

Figure 2: Soil water distribution in profile at sowing, flowering and maturity for non-TT canola (a,b,c) and TT canola (d,e,f) under three different N regimes (0, 100 and 200 kg N ha^{-1}) in 2011.where horizontal bars show the standard error for the measured value.

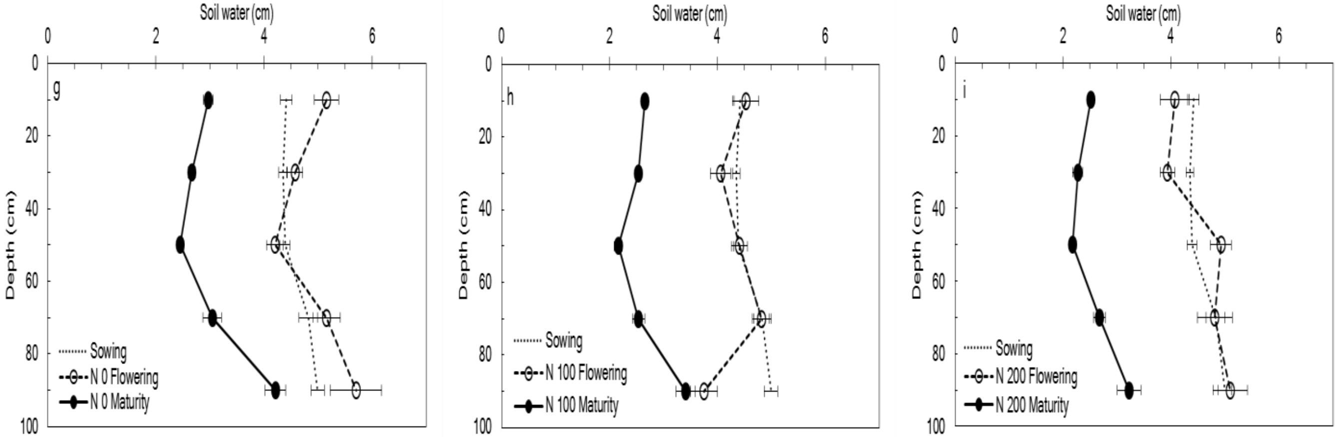

Figure 2 (cont.): Soil water distribution in profile at sowing, flowering and maturity for mustard (g,h,i) under three different N regimes (0, 100 and 200 kg N ha^{-1}) in 2011.where horizontal bars show the standard error for the measured value.

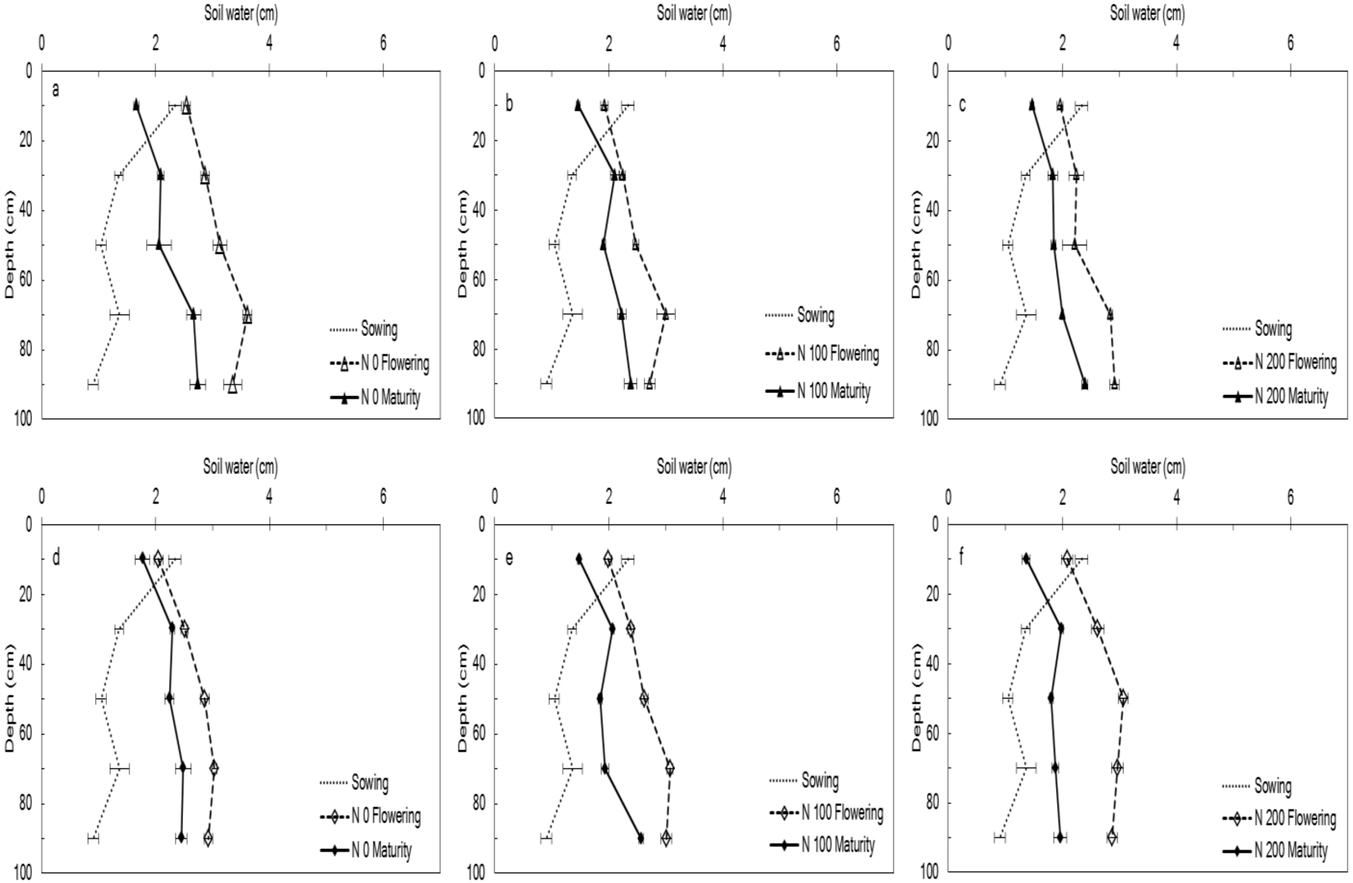

Figure 3: Soil water distribution in profile at sowing, flowering and maturity for non-TT canola (a,b,c) and TT canola (d,e,f) under three different N regimes (0, 100 and 200 kg N ha^{-1}) in 2012. where horizontal bars show the standard error for the measured value.

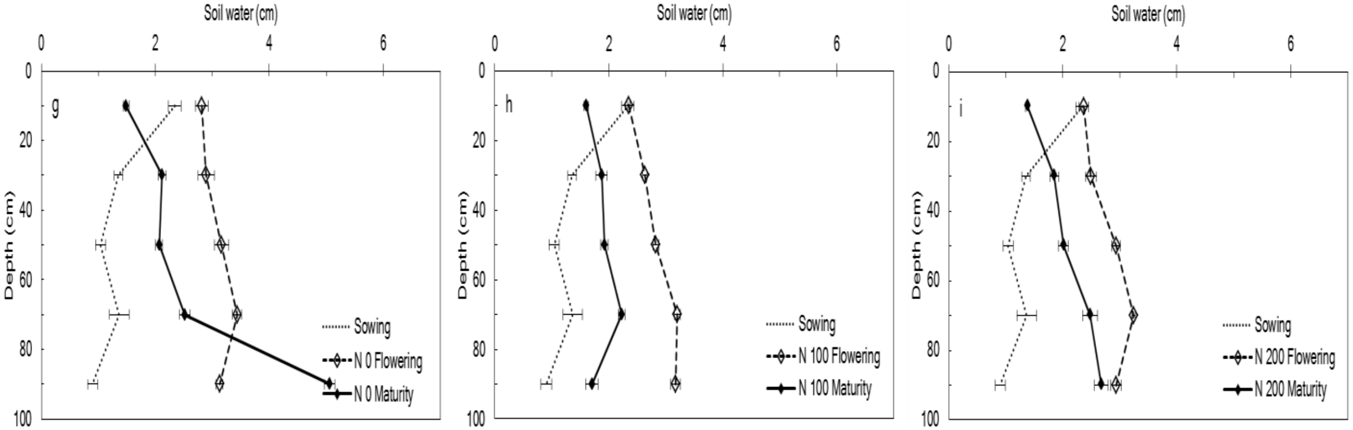

Figure 3 (cont.): Soil water distribution in profile at sowing, flowering and maturity for mustard (g,h,i) under three different N regimes (0, 100 and 200 kg N ha^{-1}) in 2012. where horizontal bars show the standard error for the measured value.

In contrast to 2011, a very dry summer and autumn in 2012, resulted in the soil profile at sowing being extremely dry (Fig: 3) consequently, there was some accumulation of soil water after rainfall was received in winter. Surprisingly, canola (both TT and non-TT) and mustard were not able to dry the soil profile down to levels present at sowing. There was less water accumulation at 70-90 cm in non-TT cultivars in 100 kg N ha^{-1} and 200 kg N ha^{-1} as compared to the control. An increase in N rate also increased extraction of soil water down the profile in TT canola (Fig: 3 d-f). There was large accumulation of soil water at 90 cm depth in mustard where no fertiliser N was applied. This build-up in soil water at depth was not evident in 100 kg N ha^{-1} or 200 kg N ha^{-1} (Fig: 3 g-i).

Despite all the differences in soil water extraction at mid-flowering, water extraction depth was at least 90cm at maturity for all cultivars and N treatments, expect mustard without any N, which managed to extract water from at least 70cm in 2012.

4.4.2. Nitrogen uptake and use efficiency

4.4.2.1. Nitrogen uptake and nitrogen harvest index

Total N uptake at maturity varied considerably between the two growing seasons. On average, the total N uptake by the crop at maturity in 2012 was 2.6 times lower than in 2011. Canola and mustard did not differ in N uptake during the pre- and post-flowering periods in 2011 (Table: 5). Nitrogen uptake remained low in TT cultivars during the pre- and post-flowering growing periods; hence total N uptake in TT canola at maturity was lower than that of non-TT cultivars.

In 2012, total N uptake between TT and non-TT was similar due to similar N uptake during the pre-flowering and post-flowering periods (Table: 5). There was some reduction in shoot N during the post-flowering period in 2012, which varied from 10 to 28 kg N ha^{-1} which was due to loss of leaf dry matter. Total N uptake in open pollinated cultivars was lower than the hybrid cultivars due to low N uptake during the pre-flowering period. Mustard had lower N uptake than canola during the pre-flowering period (P= 0.028) and at maturity (P=0.076).

Nitrogen application improved the total N uptake in both years. Highest N uptake was achieved with the application of 200 kg N ha^{-1} in both years followed by 100 kg N ha^{-1} and the control (Table: 6&7). Post-flowering N uptake was higher in 200 kg N ha^{-1} than all other N treatments in 2011. Nitrogen application at the rosette and green-bud stages in 2011 resulted in higher N uptake than the application of N at flowering. All other treatments that received 100 kg N ha^{-1} had similar total N uptake at maturity.

Nitrogen harvest index (NHI) of cultivars varied greatly between the seasons. In a wet season (2011) with high N post-flowering uptake, NHI was lower (0.26) than the dry season (2012) with low total N post=flowering uptake (0.40) (Table: 5). In both years, the lowest NHI was in Oasis whereas the highest was observed in Varuna. In canola cultivars, TT cultivars had a higher NHI than non-TT cultivars in 2011 but they had similar NHI in 2012.

Table 5: N uptake and NHI for different cultivars of canola and mustard during 2011 and 2012. The significance of single degree of freedom contrasts are shown: canola vs mustard (C vs M), TT canola vs non-TT canola (TT vs non-TT) and open pollinated canola vs hybrid canola (OPc vs HYc)

	N uptake (kg/ha)							
	Pre-flowering		Post-Flowering		Total		NHI	
	2011	2012	2011	2012	2011	2012	2011	2012
AV-Garnet	109.5	76.1	92.2	-10.6	201.7	65.5	0.25	0.47
Fighter TT	88.6	86.4	62.4	-27.0	151.0	59.4	0.33	0.44
Hyola555TT	83.7	108.9	67.9	-28.0	151.6	80.9	0.27	0.40
Hyola575cl	115.9	93.8	95.5	-12.2	211.4	81.6	0.26	0.46
Oasis	126.1	69.3	61.6	-9.6	187.6	59.7	0.13	0.20
Varuna	87.4	79.4	113.3	-12.0	200.7	67.4	0.33	0.49
Lsd	22.5	24.1	35.1	29.2	44.8	15.3	0.07	0.09
Orthogonal comparisons								
Cultivar	0.008	0.045	0.039	NS	0.045	0.026	<.001	<.001
C vs M	NS	0.028	NS	NS	NS	0.076	0.034	0.003
TT vs nonTT	0.004	NS	0.028	NS	0.003	NS	0.044	NS
OPc vs HYc	NS	0.025	NS	NS	NS	0.003	NS	NS

Table 6: N uptake, NHI and NUE of canola and mustard cultivars as under different N regimes during 2011. The significance of single degree of freedom contrasts are shown: N vs no N, 100 kg N ha-1 vs 200 kg N ha-1 , 100 kg N ha-1 at rosette vs 100 kg N ha-1 at green-bud or Flowering (100 R vs 100 GB/F) and 100 kg N ha-1 at green-bud vs 100 kg N ha-1 at Flowering (100 GB vs 100 F).

		N uptake kg/ha			NHI	NUE				
N Rate	Targeted GS for N	Pre-flowering	Post-Flowering	Total		N uptake efficiency	N efficiency	Agronomic efficiency	Physiological efficiency	Apparent recovery
0		69.9	53.2	123.1	0.31	1.60	19.9			
100	30,63, 71	121.5	67.8	189.3	0.25	1.07	12.3	6.5	45.0	66.1
100	51,67	110.3	87.8	198.1	0.22	1.12	11.6	5.2	21.5	74.9
100	63,71	86.0	75.1	161.1	0.24	0.91	10.0	2.4	14.3	37.9
200	30,51,63,67,71	121.7	126.9	248.6	0.28	0.90	9.0	4.8	25.5	62.7
lsd		20.1	32.3	32.6	0.03	0.23	2.3	2.4	15.9	27.1
Orthogonal comparisons										
N treatments		<.001	<.001	<.001	<.001	<.001	<.001	0.013	0.003	0.053
N vs no N		<.001	0.006	<.001	<.001	<.001	<.001			
100 kg N ha^{-1} vs 200 kg N ha^{-1}		0.059	<.001	<.001	0.01	NS	0.016	NS	NS	NS
100 R vs 100 GB/F		0.01	NS	NS	NS	NS	NS	0.013	<.001	NS
100 GB vs 100 F		0.019	NS	0.027	NS	0.071	NS	0.023	NS	0.009

Table 7: N uptake, NHI and NUE of canola and mustard cultivars under different N regimes during 2012. The significance of single degree of freedom contrasts are shown: N vs no N, 100 kg N ha^{-1} vs 200 kg N ha^{-1}, 100 kg N ha^{-1} in single application vs 100 kg N ha^{-1} in split applications (100S vs 100SP),100 kg N ha^{-1} at rosette vs 100 kg N ha^{-1} at green-bud or Flowering (100 R vs 100 GB/F) and 100 kg N ha^{-1} at green-bud vs 100 kg N ha^{-1} at Flowering (100 GB vs 100 F).

		N uptake kg ha^{-1}			NHI	NUE				
N Rate Kg ha^{-1}	Targeted GS for N	Pre-flowering	Post-flowering	Total		N uptake efficiency	N efficiency	Agronomic efficiency	Physiological efficiency	Apparent recovery
0	0	57.2	-7.9	49.3	0.41	0.43	6.94			
100	30	101.5	-36.4	65.1	0.48	0.30	4.96	3.12	44.2	15.8
100	51	99.0	-34.7	64.3	0.39	0.29	3.86	0.97	35.7	15.0
100	63	69.3	-4.3	65.0	0.44	0.30	4.39	1.97	35.3	15.7
100	30,51,63,67,71	85.6	-15.6	70.0	0.36	0.31	4.23	1.76	38.3	20.7
200	30,51,63,67,71	101.4	-0.6	100.8	0.38	0.31	3.54	1.88	39.6	25.7
Lsd		20.9	25.9	14.1	0.07	0.074	0.83	1.50	NS	13.2
Orthogonal comparisons										
N treatments		<.001	0.021	<.001	0.01	0.003	<.001	0.092	NS	NS
N vs no N		<.001	NS	<.001	NS	<.001	<.001			
100 kg N ha^{-1} vs 200 kg N ha^{-1}		NS	0.034	<.001	NS	NS	0.015	NS	NS	0.092
100S vs 100SP		NS	NS	NS	0.008	NS	NS	NS	NS	NS
100 R vs 100 GB/F		0.061	NS	NS	0.037	NS	0.024	0.014	NS	NS
100 GB vs 100 F		0.006	0.022	NS	NS	NS	NS	NS	NS	NS

In 2011, control and 200 kg N ha^{-1} had the highest NHI but NHI with 200 kg N ha^{-1} did not differ from the treatment that had a total 100 kg N ha^{-1} in three equal splits at rosette, flowering and pod development stages (Table: 6). All treatments with an application of total 100 kg N ha^{-1} had similar NHI. In 2012, higher NHI was observed in the treatments with a single application of 100 kg N ha^{-1} at rosette stage and at flowering stage than the application of 200 kg N ha^{-1} and 100 kg N ha^{-1} in five splits at key growth stages and a single application 100 kg N ha^{-1} at green-bud stage (Table: 7), but all N treatment had similar NHI compared to control.

4.4.2.2. Nitrogen use efficiency

The effect of seasonal water availability on uptake efficiency was greater than any effect of the cultivar and N treatment. In the higher rainfall season of 2011, N uptake and N use efficiency of the canola and mustard were very similar (Table: 8). Among canola cultivars, TT cultivars had lower N uptake and N use efficiency than non-TT cultivars. In drier conditions in 2012, mustard had lower N uptake and NUE than canola. All canola cultivars had similar uptake efficiency but TT cultivars had lower N use efficiency than non-TT cultivars.

Nitrogen uptake and use efficiency were significantly reduced with applications of N (Table: 6 & 7). Nitrogen uptake efficiency was not affected by the N rate whereas N use efficiency declined with increasing N rates. The N uptake efficiency was not affected by delaying the N applications in both years but N use efficiency declined with delayed N application beyond the rosette stage in drier condition of 2012.

Agronomic efficiency varied between the contrasting seasons. In a season with dry post-flowering period (2012), agronomic efficiency was less than half that observed in 2011 (1.9 kg kg^{-1} vs 4.7 kg kg^{-1}) (Table: 8).

Table 8: Nitrogen efficiencies of different cultivars of canola and mustard during 2011 and 2012. The significance of single degree of freedom contrasts are shown: canola vs mustard (C vs M), TT canola vs non-TT canola (TT vs non-TT) and open pollinated canola vs hybrid canola (OPc vs HYc)

	NUE									
	N Uptake efficiency kg ha^{-1}		N efficiency kg ha^{-1}		Agronomic efficiency		Physiological efficiency		Apparent recovery	
Cultivars	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
AV-Garnet	1.22	0.39	15.2	6.5	5.28	0.72	34.0	27.4	67.8	22.0
Fighter TT	0.91	0.30	10.8	4.0	3.70	1.60	17.1	42.8	54.4	22.5
Hyola555TT	0.91	0.40	10.2	5.1	6.20	0.62	22.6	30.6	67.6	8.0
Hyola575cl	1.29	0.37	13.9	5.6	4.19	1.51	33.6	37.9	57.6	9.4
Oasis	1.15	0.24	12.7	3.3	4.73	2.97	22.5	47.0	56.0	23.3
Varuna	1.23	0.25	12.7	3.3	4.35	4.23	29.7	46.1	58.9	26.3
Lsd	0.28	0.08	2.9	1.3	5.089	2.03	NS	NS	47.6	15.1
Orthogonal comparisons										
Cultivar	0.042	0.002	0.027	0.001	NS	0.018	NS	NS	NS	0.089
C vs M	NS	<.001	NS	<.001	NS	0.001	NS	NS	NS	0.048
TT vs nonTT	0.003	NS	0.001	0.005	NS	NS	NS	NS	NS	NS
OPc vs HYc	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.018

Agronomic efficiency was very similar across canola and mustard cultivars in 2011 but mustard had higher agronomic efficiency than canola during the dry growing conditions of 2012. Orthogonal comparisons of N treatments revealed that agronomic efficiency was higher when N was applied at the rosette growth stage than delaying N until green-bud or flowering in both years. Agronomic efficiency was higher at the green-bud stage than at flowering when N was applied at 100 kg N ha^{-1} in two splits. All other treatments were statistically similar to each other in both years.

The effect of seasonal variation on apparent recovery was greater than cultivars and N treatments; average apparent recovery was 60% in 2011 and 19% in 2012. In 2011, apparent recovery among the cultivars ranged from 55% to 68%. In 2012, canola had a lower apparent recovery than mustard possibly due to the lower apparent recovery of hybrid canola than open-pollinated cultivars (Table: 8). In 2011, delayed application of N at flowering reduced the apparent recovery more than N application at the green-bud stage. In 2012, apparent recovery was higher in 200 kg N ha^{-1} than 100 kg N ha^{-1} (P=0.092). All other treatments were similar to each other in both years.

Interestingly, physiological efficiency did not differ much during both years unlike the agronomic efficiency and apparent recovery, which varied considerably over the two years. Average physiological efficiencies were 27 kg kg^{-1} in 2011 and 39 kg kg^{-1} in 2012. Physiological efficiency among the cultivars did not differ in either year. In 2012, higher physiological efficiency was observed when a total 100 kg N ha^{-1} was applied in three splits starting at the rosette stage than N applications with similar amount in two splits at later growth stages. The amount of N applied had no effect on the physiological efficiency of canola and mustard in both years.

4.5. Discussion

In this study it became clear that soil water available for crop use has a large influence on crop water use, N uptake and NUE of canola and mustard in this Mediterranean environment. Contrasting water availabilities in the two growing seasons had a remarkable effect on water and N used by canola and mustard. Previous studies have also shown the importance of the effects of N on crop water use and vice-versa (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012). Sadras (2004) explained the degree of N and water co-limitation in wheat crop and showed that the yield gap between actual and potential yield was lower in rainfed environment when water and N equally co-limited growth of wheat. In this study the crop also responded well to N applications under high water availability but in drier conditions of 2012 crop responses to N were limited by the lower availability of water. Crop water use was higher in the wetter year (2011) than in the drier year (2012) and it had a large impact on total N uptake by canola and mustard. In the wetter year, N uptake was 2.7 times higher than in the drier year. Nitrogen uptake as a function of plant available water has been previously reported in wheat (Campbell *et al.* 2004). The effects of water availability on crop responses to N observed in this study are in agreement with Norton and Wachsmann (2006). They also showed that the small changes in crop water use had a large effect on the improvement in seed yield. Higher N uptake in wet conditions improved the N uptake efficiency, N use efficiency, agronomic efficiency and apparent N recovery by 3.5, 2.7, 2.4 and 3.5 fold than the drier conditions (2012), respectively. But the physiological efficiency and NHI were lowered in the wetter growing season by 45% and 58% when compared with the drier growing season, respectively. Improvement in N recovery from 30% to 50% with additional irrigation was also reported by Schjoerring *et al.* (1995).

The rate or timing of N applications had little effect on total water use of canola and mustard cultivars in the wetter season of 2011. These results differ from the findings of

Norton and Wachsmann (2006) who found that water use by canola and mustard cultivars was influenced by N rate in the Victorian Wimmera. However their findings are in line with the results from the 2012 growing season, in which water use of canola cultivars was more responsive to N rate than timing of application, whereas water use of mustard was more responsive to the timing of application. Mustard used less water during the pre-flowering phase than canola, which was attributed to its shorter pre-flowering duration than canola. Therefore, a greater proportion of water use in mustard was associated with dry matter accumulation during the post-flowering period. Thurling (1974) also found that mustard produced 85% of its dry matter during the post-flowering growth whereas canola produced 55% of its total dry matter during this phase.

Nitrogen treatments changed the proportion of crop water use during the pre- and post-flowering phases. On average, pre-flowering water use increased with N application in both years. Higher crop water use during the pre-flowering period in response to N application was associated with water extraction from deeper layers in the soil profile, possibly due to higher root density. Although no root data were collected in these experiments, soil water distribution in the profile at flowering supports this conclusion. Surprisingly, canola and mustard failed to dry the soil profile to levels similar to the pre-sowing soil water content in 2012 (Fig: 3). Even though canola has a deep tap-root, it failed to make use of all plant available water at depths >30 cm. Presence of high EC, boron and high pH may have been created inhospitable sub-soil at the trial sites. The clearest example of water accumulation at depth was found in mustard with no N in 2012. In this case, there was build-up in soil water at 90 cm depth between mid-flowering and maturity. It could be argued that lower shoot biomass of mustard without N was reflected in lower root growth, which limited its ability to use soil water deep in the profile. This accumulation in soil water in deeper layers of soil was not evident in treatments with 100 kg N ha^{-1} or 200 kg N ha^{-1} (Fig: 3 g-i).

In 2011, lower water use during the post-flowering period (as a %age of total water use) with 100 kg N ha^{-1} and 200 kg N ha^{-1} was attributed to improvement in crop vigour and pre-flowering water use by N (Norton and Wachsmann 2006). With the exception of mustard with no N application, all other treatments showed some extraction down to 90 cm in the soil profile.

In the higher rainfall season of 2011, N uptake by the TT canola was lower than non-TT cultivars even though their crop water use was similar. Post-flowering N uptake was also higher in non-TT cultivars, which could be related to their more vigorous shoot and root growth during early growth as water extraction depth for TT and non-TT cultivars at maturity was similar. Positive relationship between longer and vigorous root growth with higher N uptake has been reported previously (Kamh *et al.* 2005). The timing of N application at different growth stages did not influence the total N uptake, which was affected by the rate of N supply in both years. Understandably, shoot N uptake was an important determinant of crop productivity rather than the supply of N. Similar findings were reported by Cramer (1990) and (Marquard and Walker 1995).

Nitrogen uptake efficiency of cultivars under different N regimes is an important source of variation for NUE (Grami and LaCroix 1977; Yau and Thurling 1987; Möllers *et al.* 1999; Zhang *et al.* 2010). There were no cultivar × nitrogen interactions in this study for N uptake efficiency and other N use efficiency parameters. Nitrogen uptake efficiency of canola and mustard was similar in wetter conditions (2011) but mustard had lower N uptake efficiency than canola in drier conditions of 2012. Although physiological efficiency of canola and mustard did not differ during both seasons with contrasting water availabilities, mustard had lower NUE$_{SY}$ than canola. Values for physiological efficiency of 27 kg kg^{-1} in 2011 and 39 kg kg^{-1} and 2012 are similar to those reported by Smith *et al.* (1988) in rapeseed and Anderson and Hoyle (1999) in wheat for these Mediterranean environments. Physiological efficiency remained fairly stable at different N rates during both years. With the application of 100 kg N ha^{-1} at the rosette stage, physiological efficiency improved with N application compared to N applied at the green-bud stage and flowering in the wetter season (2011). However, the timing of N had no effect on physiological efficiency in the drier growing season (2012). Improvement in physiological efficiency with early application (at the rosette stage) is in agreement with the studies on wheat; the physiological efficiency of wheat improved with an application of N at GS37 (Flag leaf just visible on main stem) over N application at sowing. (Whitfield and Smith 1992; López-Bellido *et al.* 2006). TT cultivars had lower N uptake efficiency than non-TT cultivars in 2011 (higher rainfall) and lower NUE_{SY} due to similar physiological efficiency whereas in dry conditions (2012) all canola cultivars had similar N uptake and use efficiencies. In this study, N uptake efficiency decreased with application of N but was not influenced by N rate whereas NUE_{SY} decreased with increased N rate.

Nitrogen harvest index values reported here are well within the range reported for canola by Papantoniou *et al.* (2013) but much lower than the findings of (Hocking and Stapper 2001). The reason for lower NHI values in a higher rainfall season than a drier season is unclear. The decline in NHI with high N applications in the wetter season indicated inefficient use of applied N fertiliser.

Higher rates of N application and delayed N application beyond the rosette stage decreased the agronomic efficiency of canola and mustard in both years. On average, mustard had higher agronomic efficiency and apparent recovery than canola in dry conditions. These results are consistent with the study of Hocking and Stapper (2001) but lower than values reported in other studies (Smith *et al.* 1988; Hocking *et al.* 1997d).

On average, WUE did not differ between the two contrasting seasons and differences between the TT and non-TT cultivars were stable and consistent with their known differences in radiation use efficiency and transpiration efficiency (Robertson and Kirkegaard 2006). As cultivars used in this study had similar stable carbon isotope ratios, they were expected to have similar transpiration efficiency under different water availability. So in this study lower radiation use efficiency of TT cultivates than non-TT cultivars was the reason for low water use efficiency of the TT cultivars. Water use efficiency for seed yield values for canola and mustard cultivars reported here are within the range of 3-18 kg ha^{-1} mm^{-1} reported from 42 different case studies simulated by Robertson and Kirkegaard (2006). Even though WUE$_{GY}$ did not differ between the two contrasting growing seasons, the NUE in the drier season was only one-third of that observed in the wetter year (2011). A trade-off between WUE$_{GY}$ and NUE for increased N supply, as indicated by a strong negative correlation between them (In 2011, r = -1.0, n=3 and in 2012, r = -0.98, n=3), was found in this study.

4.6. Conclusion

This study has clearly shown that seasonal water availability is the main determinant of crop water use, N uptake, N use efficiency, agronomic N efficiency and apparent recovery of N in Mediterranean environment. Canola and mustard cultivars used similar amounts of water in a wetter season but canola used more water than mustard in a drier season. In 2012 (lower rainfall), water use of mustard was largely determined by the timing of N application whereas N rate was the main influence on water use by canola. Nitrogen applications did not influence the total water use of canola and mustard cultivars but changed the pre- and post-flowering partitioning of total water use. Water extraction depth at flowering was increased by the rate of N applications but all cultivars extracted soil water from similar depths at maturity. Triazine tolerant cultivars and non-TT cultivars used similar amounts of water but TT cultivars had lower N uptake, $\mathrm{NUE}_{\mathrm{SY}}$ and WUE, which could be related to their lower radiation and transpiration use efficiency. Low $\mathrm{NUE}_{\mathrm{SY}}$ in these environments was mainly related to the limitation of N uptake and low N uptake efficiency rather than physiological N efficiency. The water use efficiency of the cultivars selected for this study remained similar during contrasting availabilities of water but there were large differences in total water use between the seasons. A trade-off between NUE and WUE was observed for improved WUE with high N rates.

4.7. Acknowledgements

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Chapter 5

5. Yield dynamics of canola under different nitrogen and water regimes in South Australian Mediterranean environments

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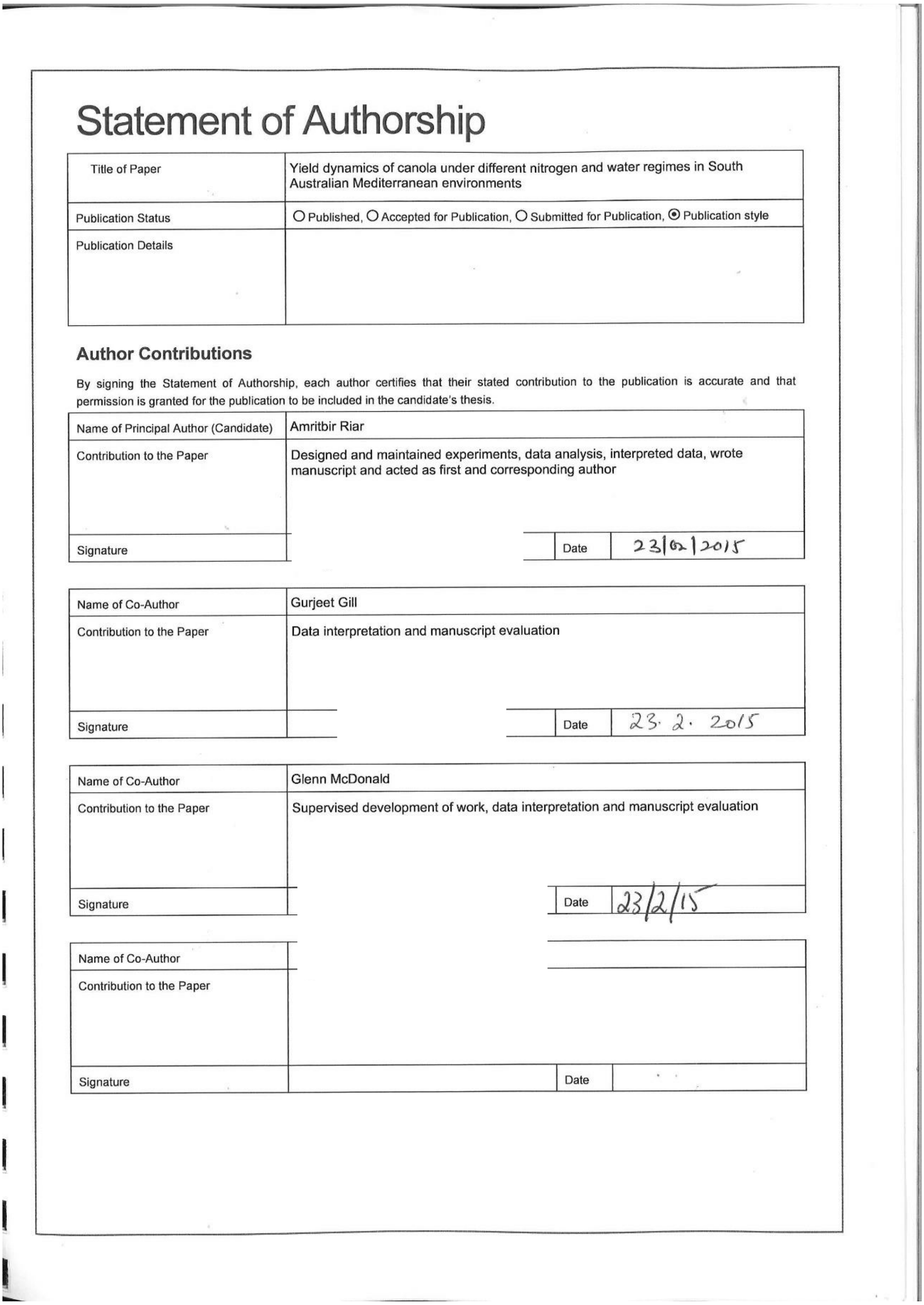

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Yield dynamics of canola under different nitrogen and water regimes in South Australian Mediterranean environments

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5.1. Abstract

Canola has a high nitrogen (N) requirement and how best to manage N in an environment where rainfall is variable is a challenging problem. Limited research has been undertaken in Australia to look at ways to improve nitrogen use efficiency (NUE) and to understand how N strategies affect canola water use (WU). The aim of this study was to investigate the impact of timing of N as split or single application at various growth stages on growth, yield and water use efficiency (WUE) under different water regimes. Field experiments were undertaken at Roseworthy (medium rainfall) and Tarlee (Mid-North High Rainfall Zone) in 2013. A medium maturity Clearfield Canola cultivar (Hyola 575cl) was sown on 17th May at Roseworthy and on 4th May at Tarlee. At each site three N rates (0, 100 and 200 kg N/ha (as granular urea) were applied after sowing when 2 leaves were unfolded (GS12) or equally split at the rosette stage (GS30), green bud appearance (GS51) and at first flower (GS60). At Roseworthy growing season rainfall was supplemented by irrigation equivalent to 60 mm of rainfall at the early rosette stage (GS30). Nitrogen application increased yield by up to 20% at Tarlee and by up to 77% at Roseowrthy, but the timing of N did not significantly affect yield. Irrigation improved total shoot dry matter at Roseworthy by 41% and yield by 49%. At both the sites there was little change in harvest index (HI) due to N and irrigation treatments and the variation in yield was strongly related to changes in crop biomass. Seedyield of canola was found to be closely associated with total dry matter production. At Roseworthy, soil water depletion was limited to a soil depth of 90 cm and at Tarlee canola used water down to 70 cm depth but at both the sites subsoil moisture was used incompletely. The average water use efficiency (WUE) of the rainfed crop at Roseworthy was 7.5 kg ha^{-1}mm^{-1}, which increased to 8.7 kg ha^{-1}mm^{-1} when the crop was irrigated. Addition of fertiliser N at this site increased the WUE_{GY} from 5.7 kg ha^{-1}mm^{-1} to 9.3 kg ha^{-1}mm^{-1}. The additional water from irrigation was used almost twice as efficiently as the seasonal water use (13.8 kg ha^{-1}mm^{-1}). WUE for yield at Tarlee was 9.3 kg ha^{-1}mm^{-1} and this was unaffected by the rate or timing of N. Nitrogen use efficiency for seed yield (NUE_{GY}) improved with irrigation at Roseworthy but decreased at the higher N rates in all water regimes. These results suggest that the rate of N rather than the timing of application was more important to yield and WUE. They also indicated that better use of subsoil moisture may be an avenue for further improvements in yield and WUE of canola in this environment.

Key words: N use efficiency, Water use efficiency, Sub-soil water use, water use patterns

5.2. Introduction

The introduction of hybrid cultivars with high early vigour, better weed control and increased use of nitrogen (N) fertilisers has improved canola yields but there is still a large gap between actual and attainable yields (Lisson et al. 2007; Kebede et al. 2010). Traditionally in southern Australian farming systems canola was grown after legume-based pastures to use high mineral nitrogen (N) in the soil and to break the cereal root disease cycle. However, with the intensification of the cropping system and increased popularity of canola, it is now often grown after cereals on soils with low N status where large application of N is required to achieve high yields. In South Australian Mediterranean environments, water and N availability are the most critical factors for sustaining crop productivity (Sadras 2002) but often water use efficiency (WUE) and N use efficiency (NUE) are low in South Australia

Campbell *et al.* (2004) stated that N yield (N uptake) is a function of plant available water as water is a major driver of yield in rainfed systems. Moreover water deficits at a critical growth stage can limit N uptake and utilisation in plants (Benjamin *et al.* 1997) and can reduce crop response to N fertilisers. In rainfed environments, water is commonly a limiting resource and its availability depends on the amount of water stored during the fallow and the amount of growing season rainfall.

Nitrogen is an expensive and difficult-to-manage input in environments with variable rainfall. Recovery of N in crops is generally less than 50% (Fageria and Baligar 2005), which cannot be justified from environmental and economic perspectives (Grant *et al.* 2002b). Canola has a high N requirement and how best to manage N in an environment where rainfall is variable is a challenging problem. The availability of N depends on mineralization of N prior to sowing the crop and within season mineralization of organic N and fertiliser N is used to apply any estimated shortfall in soil N supply In South Australian rainfed farming systems, N is generally applied prior to sowing (Parker 2009), but applying the entire N at the start of the season can be economically risky because of variable spring rainfall. Even in growing seasons with above-average rainfall, addition of all N at seedling could result in poor N efficiency by mismatching N supply with crop N demand. Nitrogen losses may occur early when crop uptake is low, N availability at later growth stages may be low when crop demand is high or high rates of N early may promote early vegetative growth and water use at the expense of later growth. Some studies have shown yield improvement with split N application over single dose of N (Ahmad *et al.* 1999; Barłóg and Grzebisz 2004b), whereas other studies found no improvement in yield with split N application as compared to its single application (Taylor *et al.* 1991; Cheema *et al.* 2001; Ahmad *et al.* 2011). It is often suggested that growers should manage fertiliser N in response to water availability and crop demand in these environments (Sadras 2004; Norton and Wachsmann 2006; Potter 2009) but usually N rate and timing are selected arbitrarily rather than based on the understanding of crop demand of N at different phenological growth stages.

The results of split and delayed applications of N are inconsistent. Some studies based on the plant N status at different growth stages showed the importance of N at the rosette to green-bud growth stages of canola (Bernardi and Banks 1993; Hocking *et al.* 1997b) whereas Dreccer *et al.* (2000a) argued that yield of oilseed rape was source limited during the pod filling stages and this limitation could be removed by adding the N at this growth stage. Moreover, based upon a simulation study, Habekotte (1997) suggested improving the source and sink capacity simultaneously for raising the potential yield of winter canola. It is widely accepted that the management of fertiliser inputs is one of the most important tools for the improvement of yield, NUE and WUE in these environments (Cooper *et al.* 1987; Angus and Van Herwaarden 2001; Sadras and Roget 2004). Consequently for this study, we hypothesise that post-sowing split applications of N at key growth stages can improve the yield, NUE and WUE of canola over its single application under different water regimes.

5.3. Materials and methods

5.3.1. Site description

Two sites with different annual rainfalls were selected to investigate the effect of N management on growth, yield, N and water use efficiency of canola. Field studies were undertaken on the Roseworthy farm (medium rainfall) of the University of Adelaide (latitude -34.53°S; longitude 138.72°E) and at a high rainfall site of the Mid-North High Rainfall Zone near Tarlee (latitude - 34.15°S; longitude 138.73°E), South Australia during the 2013 growing season. The long term annual average rainfall for the medium rainfall site is 440mm with the growing season average rainfall (defined in South

Australia as rainfall from April to October (French and Schultz 1984)) of 329 mm and the long term annual rainfall of Tarlee site is 527mm with growing season rainfall of 374mm (Fig: 1a). Annual and growing season mean maximum and minimum temperature of both sites were similar in 2013 (Fig: 1b). The main soil type at Roseworthy was a Chromosol (Isbell 2002) and medium clay over medium-heavy clay at Tarlee with an alkaline trend down the profile at both sites. In order to estimate soil moisture and nitrate-N up to a depth of 100 cm, soil cores were taken two days prior to sowing at both sites by using a 4 cm diameter hydraulic core. Soil samples were taken at five depths from across the sites at sowing, bulked, dried at 40C and sieved (<2mm) for analysis by CSBP. Starting soil moisture up to a meter depth was 198 mm at Roseworthy and 124 mm at Tarlee. Total amount of the mineral-N (ammonium + nitrate) in 0-100 cm layer was 125 kg ha^{-1} and 142 kg ha^{-1} at Roseworthy and Tarlee, respectively. Detailed soil characteristics of the experimental sites are given in Table: 1.

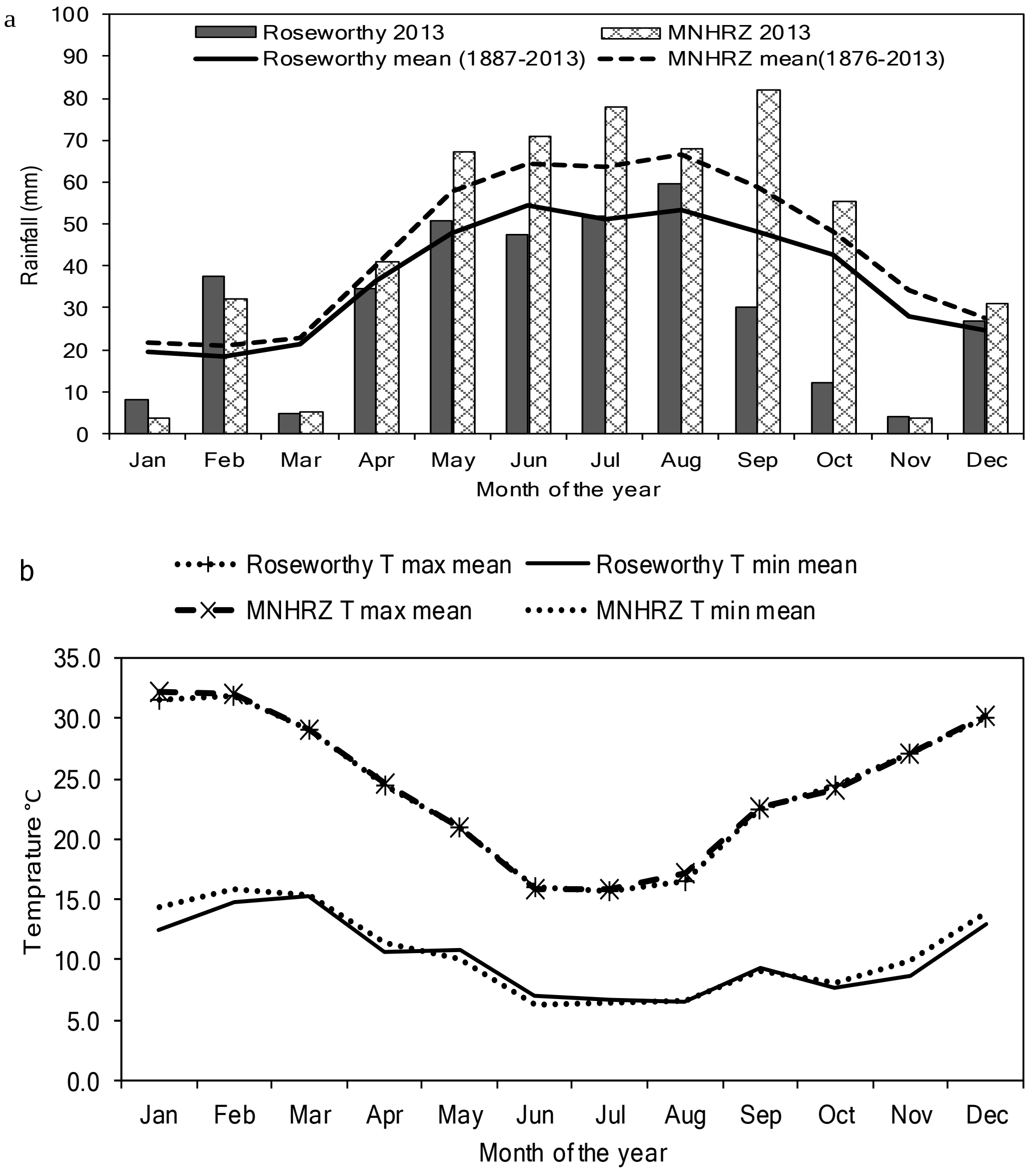

Figure 1: Long term mean and monthly rainfall, Mean minimum and maximum temperature during 2013 at Roseworthy and Tarlee sites

Table 1: Soil characteristics of each site used during 2013. Analyses were conducted by CSBP Soil and Plant Analysis Laboratories, Perth WA as explained by Rayment and Lyons (2011).

Site	Layer cm	Ammonium N (mg kg^{-1}) [21]	Nitrate N (mg kg^{-1}) [22]	Conductivity (dS m^{-1}) [23]	pH $cacl_2$	pH H_2O[24]	BD (g cm^{-3})
	0-20	8	19.0	0.285	7.3	8.2	1.2
	20-40	4	8.0	0.550	8.0	9.0	1.2
	40-60	4	0.9	1.063	8.2	9.2	1.2
RW	60-80	2	0.9	1.215	8.3	9.3	1.3
	80-100	3	1.0	1.795	8.4	9.4	1.3
	0-10	3	11.0	0.163	7.9	8.6	0.7
	10-40	1	5.0	0.195	7.9	8.8	1.0
Tarlee	40-70	< 1	4.0	0.333	8.2	9.2	1.0
	70-100	< 1	3.0	0.619	8.4	9.4	1.1

5.3.2. *Experimental design*

A medium maturity Clearfield canola cultivar (Hyola 575CL) was sown on 17th of May 2013 at Roseworthy and 4th May 2013 at Tarlee under five different N application strategies: three N rates (0, 100 and 200 kg N ha^{-1}) (as granular urea) by two timings of either all the N applied just after seedling emergence or equally split among the rosette stage (GS30), green bud appearance (GS51) and at first flower (GS60). At Roseworthy, drip irrigation was applied at the rosette stage (GS31) to wet the soil profile to the drained upper limit (DUL) to100cm depth, which was equivalent to 60 mm of irrigation. Treatments were replicated six times at Tarlee and three times at Roseworthy. The experimental design was a factorial (2 N rates x 2 timings) + added control at Tarlee and a split plot design with Irrigation was the whole plot and the N treatments as subplots at Roseworthy. Crop growth stages were recorded by using the BBCH canola scale

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[21] Method No. 7C2b pp 130
[22] Method No. 7C2b pp 130
[23] Method No. 4A1, 4B3 & 3A1 pp 38 & 20
[24] Method No. 4A1, 4B3 & 3A1 pp 38 & 20

(Lancashire *et al.* 1991). Nitrogen was top-dressed by hand as close as possible to the desired growth stage either when the soil was wet or if rainfall was forecast within 24h after fertiliser application.

5.3.3. Crop management

The trials were sown with a cone seeder with knife point drill and press wheels at a depth of 15mm. Plots were 10 metres long comprising 6 rows with 250 mm inter-row width. Basal fertilisers were 10 kg N ha^{-1} and 11 kg P ha^{-1} as diammonium phosphate (DAP) applied at sowing and 100 kg S ha^{-1} as a pre-plant gypsum application. Seeding rates were adjusted on the basis of a germination test to achieve a plant establishment of 35 plants m^{-2}. Plant numbers were counted after crop establishment and it showed that on average there was 92% establishment at Roseworthy and 81% establishment at Tarlee Weeds were controlled by a pre-sowing application of glyphosate (2.8 L/ha) and by hand weeding in the crop when required. To avoid any early damage by insects chloropyrifos (Lorsban 700 ml/ha) was sprayed two days after sowing. Slugs and mouse damage were managed by applying snail bait (5 kg/ha) and bromadiolone (Mouse off @ 2-4 kg/ha) when necessary. Overall weed and disease incidence was minimal at both sites.

5.3.4. Measurements and sampling

Crop dry matter was measured at rosette (GS30), green-bud (GS51), 50% flowering (GS55), 50% pod development (GS75) and maturity (GS99). For measurements between GS30 and GS75 destructive samples from two rows of 50cm length (0.25 m^2) were taken. The samples were dried in an oven at 80 °C for 48 hours.

Canopy light interception measurements were taken at the same dates as the matter sampling during rosette (GS30), green-bud (GS51), 50% flowering (GS55) and 50% pod development (GS75) from 5 random locations within a plot using the AccuPar-ceptometer (Delta-T Devices Ltd.). The radiation was measured in the photosynthetically active radiation (PAR) range (400–700 nm) and measurements were taken on clear cloudless days within 2 h either side of solar noon. For each measurement, five above-canopy reading and five below-canopy readings were taken. Seasonal Radiation use efficiency (RUE) was calculated for each treatment by linear regression of sequential crop biomass measurements between the rosette (GS31) and pod-development (GS75) against intercepted PAR (Mendham *et al.* 1981). At maturity plants from two rows of 50cm length (0.25 m^2) equivalent to a half metre row were taken from each plot to estimate pod numbers per plant, seeds per pod, and harvest index (HI). The number of pods were counted on a subsample of a quarter of the inflorescence by weight from the plant sample and then converted to pod m^{-2} according to the plant density of each plot. The seed from the plant sample was threshed and. harvest index (HI) was estimated as the ratio of seed weight to total shoot weight of the subsample. Total dry matter at maturity was calculated based on plot yield and HI. Seeds pod^{-1} were estimated by counting seed from a sample of 25 pods from 5 different segments of the inflorescence, including main raceme and branches. Mean seed weight was estimated from the weight of 1000 seed. Each plot was harvested with a small plot harvester after the ends were trimmed. Seed m^{-2} calculation was based upon the seed weight and final yield.

Soil moisture content was measured pre-sowing and at maturity by using a 4cm diameter hydraulic core. For the estimation of soil moisture at sowing, 0-100cm depth of soil profile was taken at both sites, whereas at maturity 0-120cm and 0-180cm depth samples were at Roseworthy and Tarlee respectively. Cores were sub-divided into 20cm depth layers to assess the differences in water extraction at various depths. The change is soil water over 0-100cm was used to estimate crop water use (CWU) assuming no drainage below the root zone:

CWU = Growing season rainfall - (Soil moisture at harvest – soil water at sowing)

Water use efficiency (WUE) was calculated as yield divided by water used from sowing to harvest. To measure the N content of shoots, four random plants were taken from each plot at 50% flowering (GS65) and maturity (GS99), and dried in an oven at 80 °C for 48 hours. Nitrogen content was determined with a LECO combustion analyser, where plant samples were loaded into the combustion tube (at 950˚C) and flushed with oxygen. The efficiency of N for canola was calculated by using the following formulae (Fageria and Baligar 2005; Rathke *et al.* 2006):

$$\text{N use efficiency for seed yield (NUE}_{\text{GY}}\text{) (kg kg}^{-1}\text{)} = \frac{\text{yield}}{\text{N supply (Fertiliser+Soil N)}}$$

$$\text{N-uptake efficiency (kg kg}^{-1}\text{)} = \frac{\text{Total N uptake}}{\text{N supply}}$$

$$\text{Agronomic efficiency (AE)} = \frac{G_F - G_c}{F} = \text{(kg kg}^{-1}\text{)}$$

$$\text{Apparent recovery (ARE)} = \frac{N_F - N_c}{F} \times 100 = (\%)$$

$$\text{Physiological efficiency (PE) (kg kg}^{-1}\text{)} = \frac{Y_F - Y_c}{N_F - N_c}$$

$$\text{Utilization efficiency (UE)} = \text{PE} \times \text{ARE} = \text{(kg kg}^{-1}\text{)}$$

where G_F and G_C are the seed yield of the fertilised and unfertilised plots, Y_F and Y_C are the biological yield of the fertilised and unfertilised plots, N_F and N_C is the N contained in biological yield (kg ha^{-1}) of fertilised and unfertilised plots, and F was the amount of fertiliser N applied as granular urea (McDonald 1989; Fageria and Baligar 2005). Agronomic efficiency reflects the efficiency with which applied N is used and physiological efficiency can be viewed as the response of crop to additional N uptake from fertiliser. The total soil N measured at the start of the growing season was used to estimate N supply instead of seasonal N mineralisation.

5.3.5. Statistics

The data obtained from the experiment were analysed by the analysis of variance (ANOVA) using the GenStat statistical analysis software (15th edition). A Factorial + added control analysis of variance was used to identify the effects of N and water management of canola (VSN 2013). For statistical comparisons, the least significant difference (LSD) at 5% level of probability (P=0.05) was used to compare the treatments unless otherwise stated.

5.4. Results

5.4.1. Dry matter accumulation and Radiation use efficiency (RUE)

At Tarlee, canola accumulated DM right up to maturity (Fig: 2) however the application of N did not improve DM accumulation as compared to the control. Dry matter accumulation was higher after flowering in the crop supplied with fertiliser N than the control but there was no significant difference between the N rates and the time of application at any time point of measurements (Fig: 2 and Table: 2).

At Roseworthy, DM accumulation was substantially lower than at Tarlee and slowed down soon after the flowering (Fig: 2). Nitrogen did not have any effect on DM until the crop reached the pod-development stage. Total dry matter at maturity was higher in the irrigated crop than the rainfed crop (Table: 2). N application increased DM at maturity compared to the control in both irrigated and rainfed crops but N rate and timing did not influence crop DM at different growth stages.

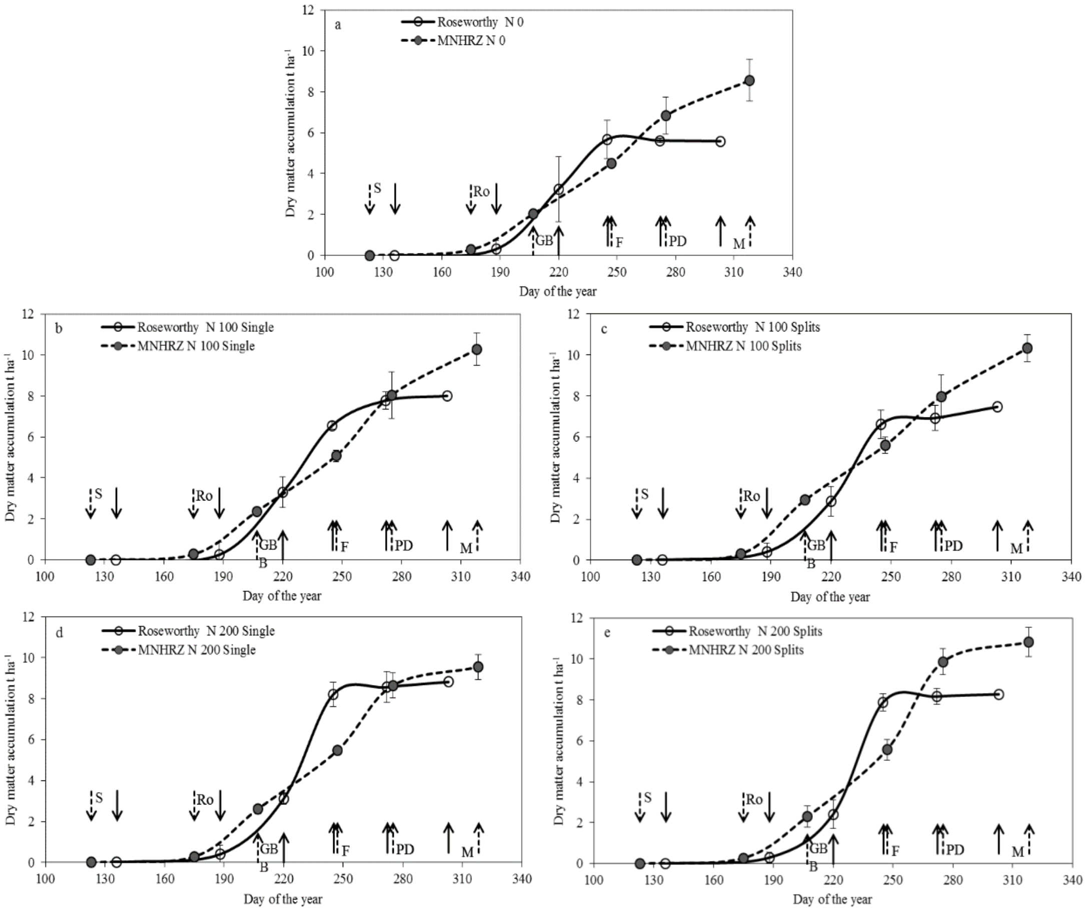

Figure 2: Dry matter accumulation with different N applications during different growth stages of rainfed canola at Roseworthy and Tarlee: (a) N 0 kg ha^{-1}, (b) N 100 kg ha^{-1} as single application (c) N 100 kg N ha^{-1} in three identical splits (d) N 200 kg ha^{-1} as single application (e) N 200 kg N ha^{-1} in three identical splits. Where letter S denote sowing, Ro is rosette, GB is green-bud, PD is pod-development and M represent physiological maturity of canola at BBCH growth scale.

Table 2: Effect of different N applications on seed yield, total dry matter (TDM), HI and oil content of canola under irrigated and rainfed conditions at Roseworthy

	N Treatments		GY (kg ha^{-1})	TDM (kg ha^{-1})	HI	Oil content (%)
Irrigation Treatment	Rate (kg ha^{-1})	Time				
I	0		1676	6228	0.27	42.8
I	100	Single	2590	10796	0.24	41.5
I	100	Split	2812	11568	0.24	42.9
Mean (100)			2701	11182	0.24	42.2
I	200	Single	3075	13376	0.23	42.4
I	200	Split	2867	12164	0.24	43.0
Mean (200)			2971	12770	0.23	42.7
RF	0		1310	5587	0.24	43.2
RF	100	Single	1745	8002	0.22	42.3
RF	100	Split	1736	7473	0.23	42.3
Mean (100)			1741	7738	0.23	42.3
RF	200	Single	1866	8812	0.21	44.1
RF	200	Split	2074	8272	0.25	42.5
Mean (200)			1970	8542	0.23	43.3
LSD $_{0.05}$						
Irrigation (I)			193	2285	NS	NS
N treatments (N)			354	1577	0.01	NS
I × N			NS	2179	NS	NS
N × Rate			NS	NS	NS	NS
N × Time			NS	NS	0.02	NS
I × N × Rate			NS	NS	NS	NS
I × N × Time			NS	NS	NS	1.4
N × Rate × Time			NS	NS	NS	NS
I × N × Rate × Time			NS	NS	NS	NS

Radiation use efficiency (RUE) of canola varied between the two sites (Table: 3). Radiation use efficiency of the crop was lower at Tarlee (1.7-2.4 g MJ^{-1}) than at Roseworthy (2.2-3.7 g MJ^{-1}). At both the sites, crop supplied with fertiliser N had greater

RUE than the control. Application of N in three splits also had slightly higher RUE than the single application of the same rate at both the sites.

Table 3: Averaged Radiation use efficiency (RUE) with different N applications between rosette (GS30) and pod-development (GS75) of canola at Roseworthy and Tarlee sites under rainfed conditions. Where Asterisks represent level of significance was <0.05 (*), <0.01 (**), and <0.001 (***).

N Treatments		RUE ($g\ MJ^{-1}$)			
		Roseworthy (rainfed)		Tarlee	
Rate ($kg\ ha^{-1}$)	Time	RUE (± s.e)	r	RUE (± s.e)	r
0		2.23 ± 0.27*	0.99	1.71 ± 0.59	0.90
100	Single	3.02 ± 0.08***	0.99	1.99 ± 0.71	0.89
100	Split	3.66 ± 0.31*	0.99	2.35 ± 0.63	0.90
200	Single	2.70 ± 0.57*	0.98	1.90 ± 0.64	0.93
200	Split	3.74 ± 0.65*	0.97	2.13 ± 1.04	0.82

Seed yield, Harvest Index and Oil content

At Roseworthy, there were no irrigation by N interactions for yield but both irrigation and nitrogen improved the yield over the rainfed and the control crop, respectively (Table: 2). The improvement of yield with N application was not due to differences in the rate or time of N application. Three way interaction between irrigation, nitrogen and timing of N application was observed for the HI and oil content (Table: 2). The application of 200 kg N ha^{-1} in three identical splits (rosette, green-bud and flowering) improved the HI over the single N application of the same amount after seedling emergence under rainfed conditions. However, no such improvement of HI was observed in the irrigated crop. Oil content was lower in the split application of 200 kg N ha^{-1} than the single N application of the same amount under rainfed conditions whereas no such reduction of oil content was observed in the irrigated crop (Table: 2).

At Tarlee N improved the yield of canola up to 100 kg/ha N but there was no effect of the timing of N application (Table: 4). Nitrogen treatments had a small effect on HI but at 100 kg N ha^{-1}, the split application treatment had a lower HI than its single application (Table: 4). The highest oil content was obtained in the control (0 kg/ha) followed by 100 kg N ha^{-1} and 200 kg N ha^{-1}, respectively (Table: 4). Within the same N application rates of 100 kg N ha^{-1} and 200 kg N ha^{-1}, three identical splits had a higher oil content than the single N application.

Table 4: Effect of different N applications on yield, total dry matter (TDM), HI and oil content of canola under rainfed conditions at Tarlee site.

N Treatments		GY (kg ha^{-1})	TDM (kg ha^{-1})	HI	Oil content (%)
Rate (kg ha^{-1})	Time				
0		2513	8555	0.28	44.7
100	Single	3021	10283	0.29	43.9
100	Split	2908	10329	0.25	44.4
Mean (100)		2965	10306	0.27	44.1
200	Single	2711	9534	0.28	43.7
200	Split	3038	10828	0.26	44.1
Mean (200)		2875	10181	0.27	43.9
LSD $_{0.05}$					
N treatments (N)		334	1463	NS	0.4
N × Rate		NS	NS	NS	NS
N × Time		NS	NS	0.03	0.4
N × Rate × Time		NS	NS	NS	NS

5.4.2. Sink development (pods m-2, seed m-2, seed pod-1 and seed weight)

At Roseworthy, the irrigated treatment produced more pods m^{-2} than the rainfed treatments (Table: 5). In irrigated treatments, the highest number of pods m^{-2} was produced with 200 kg N ha^{-1} followed by 100 kg N ha^{-1} and control, respectively. Within the same rate of N, split applications of 200 kg N ha^{-1} produced more pods m^{-2} than the single application whereas the number of pods m^{-2} with the single and split applications of 100 kg N ha^{-1} were similar.

Table 5: Effect of different N application on yield components of canola under irrigated and rainfed conditions at Roseworthy

Irrigation Treatment	N Treatments Rate (kg ha^{-1})	 Time	Pods m^{-2}× 10^{3}	Seed m^{-2}× 10^{3}	1000 Seed weight (g)	Seed pod^{-1}
I	0		4.0	44.8	3.73	12.7
I	100	Single	7.1	61.8	4.19	12.7
I	100	Split	7.7	70.6	3.98	8.0
Mean (100)			7.4	66.2	4.09	10.3
I	200	Single	10.1	79.1	3.89	13.7
I	200	Split	12.4	72.4	3.96	11.0
Mean (200)			11.2	75.7	3.93	12.3
RF	0		3.8	36.5	3.59	14.7
RF	100	Single	8.8	53.7	3.25	11.0
RF	100	Split	6.4	52.0	3.34	18.0
Mean (100)			7.6	52.8	3.30	14.5
RF	200	Single	6.0	53.2	3.51	16.0
RF	200	Split	8.7	54.3	3.82	12.0
Mean (200)			7.4	53.7	3.67	14.0
LSD $_{0.05}$						
Irrigation (I)			0.8	5.1	0.03	1.3
N treatments (N)			0.8	9.4	0.03	NS
I × N			1.0	NS	0.04	NS
N × Rate			0.8	NS	0.03	1.1
N × Time			0.8	NS	0.03	1.1
I × N × Rate			1.1	NS	0.04	1.4
I × N × Time			1.1	NS	0.04	1.4
N × Rate × Time			1.0	NS	0.04	1.2
I × N × Rate × Time			1.3	NS	NS	1.7

In the rainfed treatments, N improved the pods m^{-2} but there was no clear effect of rate and timing of N application (Table: 5). There were no interactions between the rate and timing of N for numbers of seed m^{-2} in the irrigated and rainfed crop (Table: 5). Irrigated treatments had higher seed m^{-2} as compared to the rainfed treatments. Nitrogen increased the seed m^{-2} as compared to the control in both irrigated and rainfed treatments.

The irrigated crop had a higher mean seed weight than the rainfed treatment (3.92 g of 3.52 g; Table: 5). There was a significant interaction between the rate and timing of N application. In irrigated treatments, the highest seed weight was obtained with the single application of 100 kg N ha^{-1}, which was followed by the split applications of 100 kg N ha^{-1} and 200 kg N ha^{-1}. In the rainfed treatment, the highest seed weight was observed in the split application 200 kg N ha^{-1} followed by the control, a single application of 200 kg N ha^{-1}, 100 kg N ha^{-1} in splits and a single application of 100 kg N ha^{-1}, respectively (Table: 5). Interactions for seed pod^{-1} were observed among irrigation, N application rate and time of N application. The lowest seed pod^{-1} were observed in the split application of 100 kg N ha^{-1} in the irrigated crop. On the other hand, the same treatment produced the highest seed pod^{-1} under rainfed conditions. Applications of 200 kg N ha-1 in three splits and a single application of 100 kg N ha^{-1} had similar seed pod^{-1} in rainfed and irrigated conditions. Seed pod^{-1} were higher in the rainfed than the irrigated treatments when no N was applied (Table: 5).

At Tarlee, the highest number of pods m^{-2} was observed with the single application of 200 kg N ha^{-1} (Table: 6). Application of a single dose of 100 kg N ha^{-1} improved the pods m^{-2} compared to a single application of 200 kg N ha^{-1}. Pods m^{-2} with split applications of 100 kg N ha^{-1} and 200 kg N ha^{-1} were similar to 100 kg N ha^{-1} in single application and control. In contrast, applications of N did not have a significant effect on seed m^{-2} (Table: 6). Increase in N rate from 100 to 200 kg ha^{-1} increased pods m^{-2} but reduced mean seed weight and seed pod^{-1}. A single application of 100 kg N ha^{-1} had greater seed weight than the same rate applied in splits and 200 kg N ha^{-1} and the control. Lowest seed pod^{-1} were observed in the single application of 200 kg N ha^{-1} followed by the N application of same amount in three identical splits, single application of 100 kg N ha^{-1} , 100 kg N ha^{-1} in three splits and control, respectively (Table: 6). Correlation analysis showed that seed m^{-2} was the main drive for the final gain yield (Roseworthy irrigated $r^2 = 0.99$***, Roseworthy rainfed $r^2 = 0.94$*** and Tarlee rainfed $r^2 = 0.92$***). Seed yield was strongly correlated with the seed m^{-2} through Pods m^{-2} at Roseworthy (Irrigated $r^2 = 0.67$**, Rainfed $r^2 = 0.66$***) but at Tarlee correlation between seed yield and pods m^{-2} was not significant.

Table 6: Effect of different N applications on yield components of rainfed canola at Tarlee site

N Treatments		Pods m^{-2}	Seed m^{-2}	1000 Seed weight (g)	Seed pod^{-1}
Rate (kg ha^{-1})	Time				
0		4866	94761	2.49	20.5
100	Single	7645	99877	2.84	19.5
100	Split	5710	104730	2.43	21.3
Mean (100)		6678	102304	2.63	20.4
200	Single	11320	111069	2.45	15.0
200	Split	6550	113091	2.52	17.5
Mean (200)		8935	112080	2.49	16.3
LSD $_{0.05}$					
N treatments (N)		1414	NS	0.04	0.6
N × Rate		1549	NS	0.04	0.7
N × Time		1549	NS	0.04	0.7
N × Rate × Time		1788	NS	0.05	NS

5.4.3. Water use and Water Use Efficiency (WUE)

At Roseworthy, total crop water use was higher in the irrigated crop compared to the rainfed crop (Table: 7). Irrigated canola had a higher WUE_{GY} (P=0.10) and WUE_{DM} than the rainfed crop (WUE_{GY} = 8.0 kg mm^{-1} ha^{-1}.*v* 7.2 kg mm^{-1} ha^{-1}; WUE_{DM} 33.8 kg mm^{-1} ha^{-1} *v*.31.3 kg mm^{-1} ha^{-1}). There was no effect of N rate and timing of N application on total water use. WUE_{GY} and WUE_{DM} improved with N application compared to the control but were unaffected by the N rates and timing of N application.

At Tarlee, addition of N increased total water use compared to the control. Lack of effect of the rate and timing of N application on total water use indicated that all N treatments used similar amount of water from sowing to maturity (Table: 8). Water use efficiency for yield (WUE_{GY}) and water use efficiency for dry matter (WUE_{DM}) did not vary among the N treatments.

Table 7: Effect of different N applications on water use, water use efficiency, N uptake efficiency, N use efficiency for seed yield and nitrogen harvest index (NHI) of canola under irrigated and rainfed conditions at Roseworthy

	N Treatments								
Irrigation Treat.	Rate (kg ha^{-1})	Time	WU (mm)	WUE$_{GY}$ (kg mm^{-1} ha^{-1})	WUE$_{DM}$ (kg mm^{-1} ha^{-1})	Total N uptake (kg ha^{-1})	N up efficiency (kg kg^{-1})	NUE$_{SY}$ (kg kg^{-1})	NHI
I	0		293	5.8	21.4	151	1.21	13.41	0.38
I	100	Single	301	8.6	35.9	253	1.12	11.51	0.34
I	100	Split	294	9.6	39.3	210	0.93	12.50	0.44
Mean (100)			298	9.1	37.6	231	1.03	12.00	0.39
I	200	Single	314	10.0	43.6	283	0.87	9.46	0.36
I	200	Split	294	9.7	41.3	240	0.74	8.82	0.38
Mean (200)			304	9.9	42.5	261	0.80	9.14	0.37
RF	0		235	5.7	24.1	111	0.89	10.48	0.39
RF	100	Single	232	7.6	34.8	180	0.80	7.76	0.34
RF	100	Split	226	8.0	34.5	169	0.75	7.72	0.35
Mean (100)			229	7.8	34.7	174	0.77	7.74	0.34
RF	200	Single	240	7.8	36.8	179	0.55	5.74	0.31
RF	200	Splits	253	8.5	33.6	174	0.54	6.38	0.40
Mean (200)			247	8.2	35.2	176	0.54	6.06	0.35
LSD $_{0.05}$									
Irrigation (I)			54	NS	2.1	38	0.24	0.82	NS
N treatments (N)			NS	1.5	6.6	43	0.23	1.741	NS
I × N			NS	NS	NS	NS	NS	NS	NS
N × Rate			NS	NS	NS	NS	NS	NS	NS
N × Time			NS	NS	NS	NS	NS	NS	NS
I × N × Rate			NS	NS	NS	NS	NS	NS	NS
I × N × Time			NS	NS	NS	NS	NS	NS	NS
N × Rate × Time			NS	NS	NS	NS	NS	NS	NS
I × N × Rate × Time			NS	NS	NS	NS	NS	NS	NS

.

Table 8: Effect of different N applications on water use, water use efficiency, N uptake efficiency, N use efficiency for seed yield and nitrogen harvest index (NHI) of rainfed canola at Tarlee site.

N Treatments								
Rate (kg ha^{-1})	Time	Total WU (mm)	WUE_{GY} (kg mm^{-1} ha^{-1})	WUE_{DM} (kg mm^{-1} ha^{-1})	Total N uptake (kg ha^{-1})	N up efficiency (kg kg^{-1})	NUE_{SY} (kg kg^{-1})	NHI
0		294	8.53	29.0	179	1.26	17.7	0.42
100	Single	311	9.72	33.1	237	0.98	12.5	0.39
100	Split	305	8.92	34.0	231	0.95	11.2	0.35
Mean (100)		308	9.32		234	0.97	11.8	0.37
200	Single	309	9.47	31.0	236	0.69	8.5	0.38
200	Split	312	9.75	35.0	247	0.72	8.9	0.38
Mean (200)		310	9.61		241	0.71	8.7	0.38
LSD $_{0.05}$								
N treatments (N)		10	NS	NS	41	0.22	1.7	NS
N × Rate		NS	NS	NS	NS	0.24	1.9	NS
N × Time		NS	NS	NS	NS	NS	NS	NS
N × Rate × Time		NS	NS	NS	NS	NS	NS	NS

5.4.4. Water distribution in the soil profile

At Tarlee, there was > 3cm of water in all 20 cm depth increments up to 60 cm at the time of sowing whereas at Roseworthy soil up to 40 cm had <3 cm of water for each measured increment but deeper layers had > 3cm of soil water at sowing. At maturity at both the sites, application of 200 kg N ha^{-1} and 100 kg N ha^{-1} dried the profile more than no nitrogen treatment (Figure 3). At Tarlee, the depth of water extraction was at least 90 cm with and without N application (Figure 3a, b, c, d, e) and all treatments dried the profile to the lower limit (LL) at 70cm. However there was more water available water at depths below the 70 cm. At Roseworthy, canola extracted soil water to 70 cm in the irrigated and at least to 50 cm in the rainfed treatment (Figure 3 f, g, h, i, j & 4 k, l, m, n, o). N management did not appear to influence the depth of water extraction by canola. In the irrigated treatment, the crop dried the profile to the LL up to the depth of 50 cm in the control and single application of 100 kg N ha^{-1} and 200 kg N ha^{-1} (3f, g, i). However, in the split N application soil profile was not dried to the LL at any measured depth in irrigated treatments (3h, j). In contrast, soil profile was dried down to LL at the depth up to 50cm in all rainfed treatments (Fig: 3k, l, m, n, o).

5.4.5. Nitrogen uptake, N uptake efficiency and Nitrogen harvest index

At Roseworthy, irrigation and N application improved the total N uptake, N uptake in seed and N uptake efficiency without interacting with each other (Table: 7). There was no effect of rate and timing of N application on total N uptake, N uptake in seed and N uptake efficiency. NHI was not affected by irrigation and N treatments (Table: 7).

At Tarlee, total N uptake and N uptake in seed were improved by the N treatments but there was no significant difference between the rate and timing of N (Table: 8). Nitrogen uptake efficiency was the lowest at 200 kg N ha^{-1} and highest for the control. Split application of N did not increase N uptake efficiency relative to the single application. Nitrogen harvest index (NHI) slightly decreased with the addition of N (P=0.10) but N treatments did not differ among each other for NHI (Table: 8).

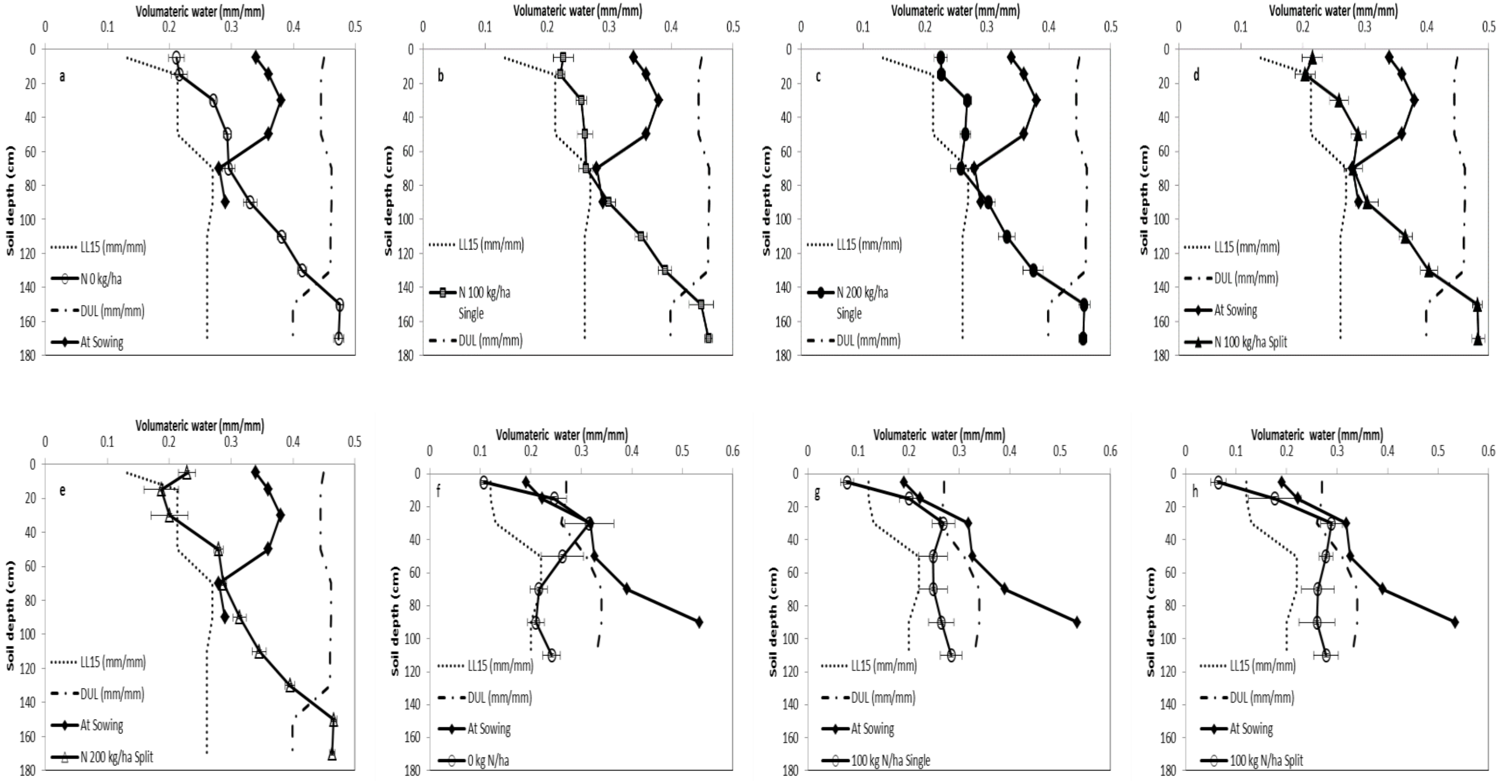

Figure 3: Soil water distribution in profile at sowing and maturity for Tarlee rainfed (a,b,c,d,e), Roseworthy irrigated (f,g,h,i,j) and Roseworthy rainfed (k,l,m,n,o) for different N regimes, Where LL15 and DUL are the lower limit and drainage upper limit for water extraction

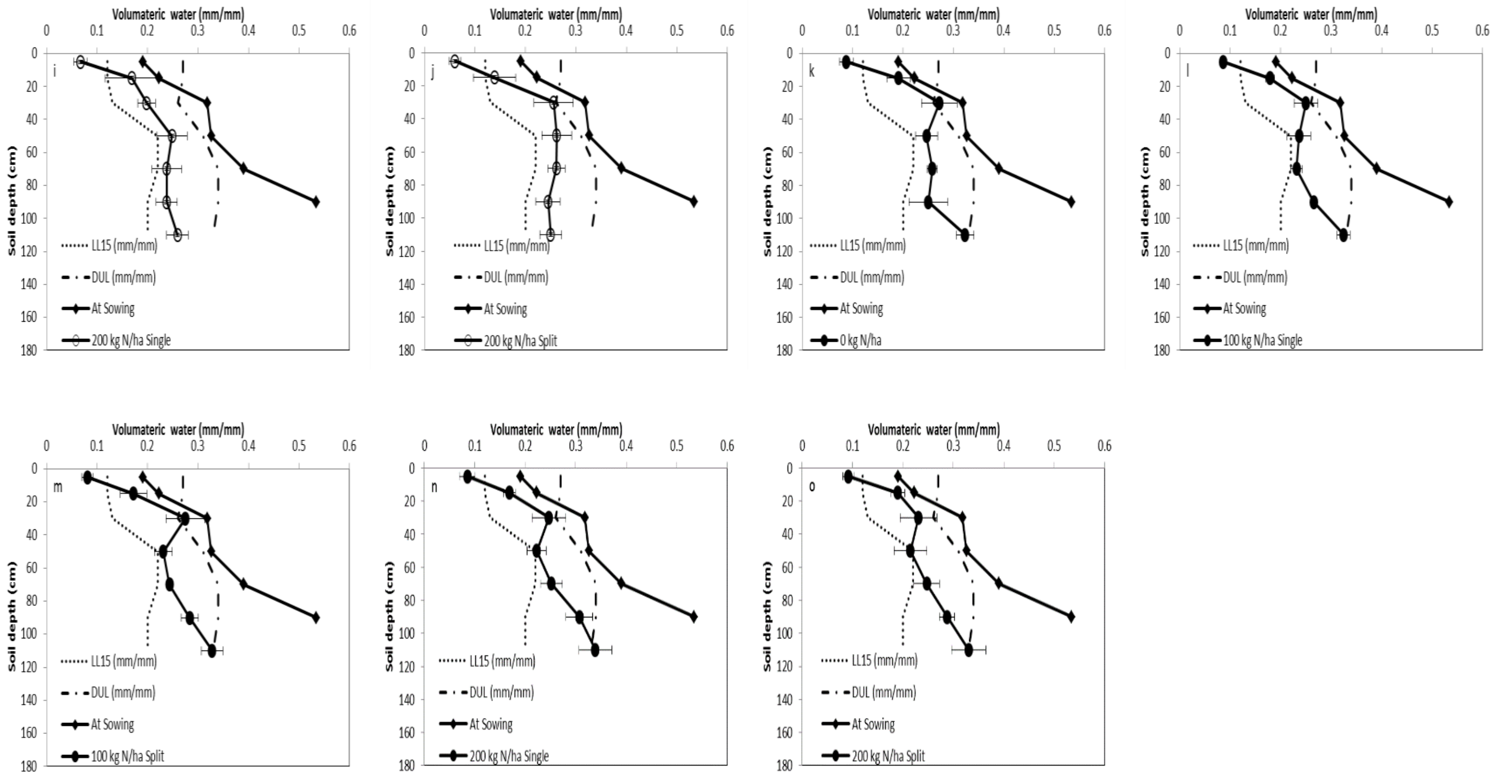

Figure 3(cont.): Soil water distribution in profile at sowing and maturity for Tarlee rainfed (a,b,c,d,e), Roseworthy irrigated (f,g,h,i,j) and Roseworthy rainfed (k,l,m,n,o) for different N regimes, Where LL15 and DUL are the lower limit and drainage upper limit for water extraction

5.4.6. Nitrogen use efficiency

At Roseworthy, irrigation and N applications improved the N use efficiency for seed yield. The NUE for seed yield decreased with increasing rate of N application (Table: 7). There was no significant difference between the single and split application of N for NUE_{GY}. In the irrigated treatment, agronomic efficiency decreased at higher N rate whereas there was no effect of N rate in rainfed treatments (Table: 9). Split applications of N had similar agronomic efficiency as with single application of 100 kg N ha^{-1} and 200 kg N ha^{-1} in both irrigated and rainfed treatments. Apparent recovery and physiological efficiency was not influenced by irrigation or N but utilization efficiency was higher for the irrigated crop than rainfed (Table: 9). In irrigated treatments, N utilization efficiency was higher with 100 kg N ha^{-1} compared to the application of 200 kg N ha^{-1} whereas it was similar for 100 kg N ha^{-1} and 200 kg N ha^{-1} in the rainfed treatment.

Table 9: Effect of different N applications on agronomic efficiency, physiological efficiency, apparent recovery, utilization efficiency and agro-physiological efficiency of canola under irrigated and rainfed conditions at Roseworthy

	N Treatments		Agronomic efficiency (kg kg^{-1})	Physiological efficiency (kg kg^{-1})	Apparent recovery (%)	Utilization efficiency (kg kg^{-1})	Agro-physiological efficiency (kg kg^{-1})
Irrigation Treat	Rate (kg ha^{-1})	Time					
I							
I	100	Single	9.14	56	82.3	54.8	9.1
I	100	Split	11.36	64	58.6	64.8	31
Mean (100)							
I	200	Single	6.99	73	65.9	42.7	11.1
I	200	Split	5.96	87	44.5	35.6	14.8
Mean (200)							
RF	0						
RF	100	Single	4.35	83	45.6	28.5	13.8
RF	100	Split	4.26	37	57.5	23.1	6.8
Mean (100)							
RF	200	Single	2.78	69	33.9	18.9	10.3
RF	200	Split	3.82	72	31.5	17.2	17.3
Mean (200)							
LSD $_{0.05}$							
Irrigation (I)			1.819	NS	NS	10.47	NS
I × Rate			2.572	NS	NS	14.8	NS
I × Time			NS	NS	NS	NS	NS
I × Rate × Time			NS	NS	NS	NS	NS

At Tarlee, NUE for seed yield decreased as N rate increased from 100 to 200 kg ha^{-1} but there was no effect of the timing of N application (Table: 8). The highest agronomic efficiency of 5.1 kg kg^{-1} was achieved with the single application of 100 kg N ha^{-1} whereas all other N treatments have similar agronomy efficiency (Range: 2.0-2.6 kg kg^{-1}) (Table: 10). Apparent recovery of N decreased at high N rates but within the same rate of N application, splitting N did not improve the apparent recovery over single N application. Physiological efficiency did not differ among N treatments and the control.

Nitrogen utilization efficiency decreased as N rate increased from 100 to 200 kg ha^{-1}, especially at the lower rate of N (Table: 10).

Table 10: Effect of different N applications on agronomic efficiency, physiological efficiency, apparent recovery, utilisation efficiency and agro-physiological efficiency of rainfed canola at Tarlee site

N Treatments Rate (kg ha^{-1})	 Time	Agronomic efficiency (kg kg^{-1})	Physiological efficiency (kg kg^{-1})	Apparent recovery (%)	Utilization efficiency (kg kg^{-1})	Agro-physiological efficiency (kg kg^{-1})
100	Single	5.1	32.6	58.2	22.4	10.9
100	Split	2.0	40.9	51.6	19.7	5.8
Mean (100)		3.5	36.8	54.9	21.1	8.4
200	Single	2.0	37.7	28.5	6.9	9.6
200	Split	2.6	48.1	33.9	14.0	8.3
Mean (200)		2.3	42.9	31.2	10.5	9.0
LSD $_{0.05}$						
N × Rate		NS	NS	21.2	8.5	NS
N × Time		NS	NS	NS	NS	NS
N × Rate × Time		2.3	NS	NS	NS	NS

5.5. Discussion

This study showed that yield was driven mainly by the biomass production (Roseworthy: *r* = 0.99; Tarlee: *r* = 0.97). Responses in yield were affected mainly by changes in crop dry matter because there was very little difference in the HI among the treatments or between the two trial sites. On average canola converted around 24% of its biomass into yield at Roseworthy and around 27% at Tarlee. Interestingly, irrigation at the rosette stage at Roseworthy improved yield by 49% with an increase of 41% in total shoot dry matter without any considerable improvement in HI.

Independence of yield from HI in canola observed in this study was also reported for different B*rassica* species by Lewis and Thurling (1994). Improvement in dry matter production with N from flowering to maturity at the high rainfall site and from pod development to maturity at Roseworthy was observed under rainfed conditions. Thus N improved the total dry matter production and seed yield at both sites but there was no significant effect of N rate and timing of N application. Moreover the effect of limited N uptake on dry matter production was additive to critical effect of shading by flowering and pods. According to Diepenbrock (2000), flowering is the most critical stage to influence canola yield even under favourable conditions, due to decrease in the total leaf area by shading, firstly by onset of flowering and then by pods (Gabrielle *et al.* 1998).

Differences in total dry matter and yield between the two sites and different water regimes were attributed not only to water availability but also to the different rainfall patterns. The high rainfall site received around 30% more rainfall than Roseworthy from sowing to mid-flowering period (May-August) but rainfall was 2.5 times higher during the late-flowering and pod initiation period (September) and around 3 times higher during the pod-development phase (October) (Fig: 1). The lower dry matter and yield at Roseworthy compared to Tarlee in rainfed conditions may have been due to water stress during flowering and pod-development phases, which may have reduced crop dry matter production, N uptake and utilisation (Sadras 2004; Sinclair and Rufty 2012). Richards and Thurling (1978) also found significant reduction in canola dry matter and yield components due to water stress at flowering and pod development.

The mean oil concentration for canola was greater than 42% at both the sites. Oil concentration in canola seed was unaffected by the site or irrigation regime at Roseworthy. Nitrogen application slightly reduced the oil content of seed at the high rainfall site compared to the control, which could be due to the inverse relationship between oil and protein as reported in the literature (Taylor *et al.* 1991; Zhao *et al.* 1993; Hocking *et al.* 1997f). Split applications of N improved the oil content as compared to the single N application at the high rainfall site but the difference between the treatments was <0.5%. At Roseworthy, split application improved seed oil content in irrigated conditions but not in rainfed environment. High oil content in the irrigated crop at Roseworthy may be attributed to a longer period of seed development and slower seed maturity than in the rainfed crop due to higher water availability.

Seed yield formation involves complex interactions between various yield components. Several studies have reported pod number to be the main factor responsible for yield (Scott *et al.* 1973; Beversdorf *et al.* 1988; Wright *et al.* 1988; Hocking *et al.* 1997d). Several studies have also shown that pods per plant and pods m^{-2} can be strongly influenced by a number of developmental and environmental factors as well as the supply of water and nutrients (Allen and Morgan 1972; Tayo and Morgan 1975; Diepenbrock 2000): In this study, water was not the main factor affecting the pods m-2 (range: 6.7-8.2 × 10^3) but applications of N improved pods m^{-2} by 60% at Tarlee, 98% at Roseworthy in rainfed conditions and by 233% at Roseworthy in irrigated conditions. Pods m^{-2} were strongly correlated with yield at Roseworthy (rainfed r = 0.81; irrigated r = 0.82; n = 15) whereas yield was not correlated with pods m^{-2} at Tarlee. At all the sites, seed m^{-2} were more strongly correlated with the yield than pods m^{-2} (r = 0.96, 0.97 and 0.99 for Tarlee (n = 30), Roseworthy rainfed (n = 15) and irrigated (n = 15), respectively). Nitrogen improved the number of seed m^{-2} up to 100 kg N ha^{-1} but higher rate or the timing of N application did not influence this trait.

Irrigation at Roseworthy had a large influence on crop water use and N uptake of canola crop. Previous studies have also shown large effects of N on crop water use and vice-versa (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012).

Sadras (2004) explained the degree of N and water co-limitation in wheat crop and showed that the yield gap between actual and potential yield was lower in rainfed environment when water and N equally co-limited the growth of wheat. Nitrogen application did not influence total crop water use at Roseworthy. However, at the high rainfall site canola used 15 mm more water when N was applied but there was no difference between 100 kg and 200 kg N ha^{-1} treatments. Average WUE for yield was 7.5 kg ha^{-1}mm^{-1} and 8.0 kg ha^{-1}mm^{-1} at Roseworthy and high rainfall site, respectively. Overall, WUE$_{GY}$ was not significantly affected by N rate or application timing. NUE$_{GY}$ was significantly reduced at high N rates at both the sites but the timing of N application had no impact. The values of WUE for yield reported here are within the range of 3-18 kg ha^{-1} mm^{-1} reported from 42 different case studies simulated by Robertson and Kirkegaard (2006). At Roseworthy, irrigation at the rosette stage improved WU and WUE of canola. The average WUE of the rainfed crop was 7.5 kg ha^{-1}mm^{-1} which increased to 8.7 kg ha^{-1}mm^{-1} in the irrigated crop. The irrigated treatment used 62 mm more water than the rainfed treatment. The additional water from irrigation was used almost twice as efficiently as the seasonal water use (WUE = 13 kg ha^{-1}mm^{-1}).

Dependence of N uptake on plant available water has been previously reported in wheat (Campbell *et al.* 2004): This was also the case in this study and the observed effects of water availability on crop response to N are in agreement with Norton and Wachsmann (2006). They also showed that the small changes in crop water use had a large effect on the improvement in yield in canola. The water use, WUE$_{GY}$, WUE$_{DM}$, total N uptake, N uptake efficiency and NUE$_{SY}$, were similar for canola grown at the high rainfall site (Tarlee) and with irrigation at Roseworthy (RW). NHI was similar at both site but on average values for agronomic efficiency, physiological efficiency, apparent recovery and utilization efficiency were lower for the high rainfall site than

Roseworthy. Nitrogen harvest index values reported here are well within the range reported for canola by Papantoniou *et al.* (2013) but much lower than the findings of Hocking and Stapper (2001) from Australia. The decline in NHI at high N rates at the high rainfall site indicated inefficient use of applied N fertiliser.

At Roseworthy, higher crop water use in irrigated crop improved N uptake by 1.4-fold as compared to the rainfed crop, which improved N uptake efficiency and N use efficiency by 40%. Furthermore, irrigation at Roseworthy had 2.2 and 2.3 fold greater agronomic efficiency and N utilization efficiency, respectively. Physiological efficiency, apparent recovery and NHI were unaffected by the irrigation application at this site at the rosette stage.

The timing of N application (single vs split) of 100 kg N ha^{-1} and 200 kg N ha^{-1} had no effect on total crop water use, WUE_{GY}, $WUE_{DM,}$ total N uptake, N uptake efficiency, NUE_{SY} and NHI. It appears crop demand was satisfied by the application of 100 kg N ha^{-1}. In this study, N rates significantly increased crop water use at Tarlee but not at Roseworthy under rainfed conditions. Therefore, our results differ somewhat from Norton and Wachsmann (2006) who found that water use by canola and mustard cultivars was influenced by N rate in the Victorian Wimmera.

As argued earlier, differences between the responses of canola to N under different water regimes were mainly due to the rainfall pattern rather than water availability. This is also evident from the unused water left in soil profiles after crop harvest. As on average, around 90, 86 and 84 mm unused water above crop lower limit was present in the soil profiles after crop harvest at the high rainfall site, medium rainfall site rainfed conditions and medium rainfall site irrigated, respectively. Moreover canola crops extracted water down to 70-90 cm depth at maturity in all N and non-N treatments but additional N dried the profile more by maturity compared to no N with little effect on total water use. Most of the unused water remained at 30-70 cm depth, which indicated that better use of subsoil moisture may be an avenue for further improvements in yield and WUE canola.

5.6. Conclusions

This study revealed that the rates (100 kg N ha^{-1} and 200 kg N ha^{-1}) and timing (single and splits) of N had little impact on yield, total dry matter, water use and WUE. This indicated that N application of 100 kg ha^{-1} adequately supplied crop demand for N during the growing season of 2013. At Roseworthy, drip irrigation was used to saturate the soil profile at the early rosette stage, which was equivalent to 60 mm of rainfall. N application increased yield by up to 20% at Tarlee and by up to 77% at medium rainfall site, but the timing of N did not significantly affect yield. Irrigation improved total shoot dry matter by 41% and yield by 49%. At both the sites there was little change in HI due to N and irrigation treatments and the variation in yield was strongly related to changes in crop biomass. Seed yield of canola was found to be closely associated with total dry matter production and seed m^{-2}. At Roseworthy, soil water depletion was limited to a soil depth of 90 cm and at Tarlee canola used water down to 70 cm depth but at both the sites subsoil moisture was used incompletely. The average WUE of the rainfed crop at Roseworthy was 7.5 kg ha^{-1}mm^{-1}, which increased to 8.7 kg ha^{-1}mm^{-1} when the crop was irrigated. Addition of fertiliser N at this site increased the WUE$_{GY}$ from 5.7 kg ha^{-1}mm^{-1} to 9.3 kg ha^{-1}mm^{-1}. The additional water from irrigation was used almost twice as efficiently as the seasonal water use (13.8 kg ha^{-1}mm^{-1}). WUE for yield at Tarlee was 9.3 kg ha^{-1}mm^{-1} and this was unaffected by the rate or timing of N. Nitrogen use efficiency for seed yield improved with irrigation at Roseworthy but decreased at the higher N rates in all water regimes These results suggest that the rate of N rather than the timing of application was more important to yield and WUE. It also indicated that better use of subsoil moisture may be an avenue for further improvements in yield and WUE of canola in this environment.

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Chapter 6

6. Effect of post-sowing nitrogen management on Co-limitation of nitrogen and water in canola and mustard

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Contribution to the Paper	Supervised development of work, data interpretation and manuscript evaluation		
Signature		Date	23/2/15

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Effect of post-sowing nitrogen management on Co-limitation of nitrogen and water in canola and mustard

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6.1. Abstract

Nitrogen (N) and water are the key factors limiting maximum attainable yield (Ya) in semiarid and arid Mediterranean environments. Initially based upon the Bloom's economic analog for resource limitations, the hypothesis of negative relationship to the degree of water and N co-limitation (CWN) with the yield gap (Yg, defined as the difference between actual and attainable seed yield) has been supported by some empirical and simulation studies. However, all the work so far on water and N co-limitation has been done in cereals (determinate crops) with a fixed amount of N applied pre-sowing whereas in rainfed environments post-sowing nitrogen management is an important tool for risk management and for improved nitrogen use efficiency. The aim of this work was to test the concept of water and N co-limitation with post-sowing nitrogen management in the indeterninant crops canola (*Brassica napus*) and mustard (*B. juncea*). Three field experiments with different cultivars of canola and mustard were undertaken under different water regimes. Three N rates (0, 100 and 200 kg N ha^{-1} as granular urea) applied at different growth stages sowing were used to evaluate different N management strategies. Seasonal water stress index (*WSI*) and nitrogen stress index (*NSI*) were estimated and the total stress ($T_{WN;}$ sum of WSI and NSI), maximum stress ($M_{WN;}$ maximum of WSI and NSI) and the co-limitations of water and N stress were calculated. This study provides the first empirical evidence that yield of canola and mustard is co-limited by water and nitrogen under the post-sowing nitrogen application.

Yield gaps were more strongly related with the *NSI* than *WSI. WSI* was found to be negatively associated with the spring rainfall whereas *NSI* was inversely related to the growing season rainfall. Both T_{WN} and M_{WN} stress indices were also negatively related with yield gap and WUE. Yield gaps reduced with the increased degree of water and N co-limitation, either expressed by CT_{WN} or CM_{WN}. Average yield gap reduction of 1.1 t ha^{-1}was observed with greater N-water co-limitation (Range 0.5- 2 t ha^{-1}). Seasonal variation in in the reduction of yield gap with the degree of co-limitation was observed for both CT_{WN} and CM_{WN}. No relation was found between $\mathrm{NUE}_{\mathrm{SY}}$ of canola and mustard and any of the stress and co-limitation indices. The analysis showed that N is a bigger limiting factor for seed yield than water, which may be due to low N uptake efficiency during the pre-flowering period and low physiological efficiency during the post –flowering period. Crop response to N was reduced by water stress with low spring rainfall as even applying higher rate of N at five key growth stages did not reduce the yield gap. Future studies need to focus on the interaction of pre and post-flowering WSI and NSI in devising crop management tools for this environment.

6.2. Introduction

The introduction of improved cultivars of canola and mustard with high early vigour, better weed control options and increased use of N fertilisers have improved seed yields of canola and mustard. However, there is still a large gap between actual and attainable yields of canola and mustard (Lisson *et al.* 2007; Kebede *et al.* 2010). As nitrogen (N) and water are the key factors limiting crop yield under Mediterranean environments (Cossani *et al.* 2010), it will be difficult to realise the full potential of genetic improvement without improving the N and water use of these crops (Sinclair and Rufty 2012).

Water availability has a large influence on crop demand for and its response to N. The availability of N depends on pre-sowing mineralization of N and within season mineralization of organic N, whereas water is always a limiting resource in rain-fed systems and its availability depends on the total rainfall during the fallow and growing season which vary seasonally. Effective water use is vital to minimise the gap between actual and maximum attainable water-use efficiency (WUE) under rain-fed systems (Sadras and Angus 2006) hence effective water use can reduce the yield gap (defined as the difference between actual and attainable seed yield). Total N uptake is also a function of plant available water (Campbell *et al.* 2004) and water deficits can limit N responses by reducing N uptake and utilisation (Benjamin *et al.* 1997). Many studies have shown these effects of water availability on N response of crops and *vice-versa* (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012).

Bloom *et al.* (1985) proposed that plants control the allocation of resources so that growth is equally limited by all resources. Consequently, growth of the plant would be optimum when all resources are equally limiting. In other words, the growth and yield of a crop stressed with scarcity of N and/or water will be positively related to the degree of co-limitation of these resources. Presence of co-limitation has been identified in different systems; from cell to biomes (Venterink *et al.* 2001; Flynn 2002; Maberly *et al.* 2002). Several studies identified that co-limitation in a system arises from and is influenced by several mechanisms, including the interaction between the different components and factors of the system, e.g. chronological impact of factors at different time scale and influence of different factors on different system components . The degree of co-limitation changes over time with availability and interactions of different resources (Sinclair and Park 1993; Berman and DeJong 1997; Maberly *et al.* 2002; Sadras 2004). Sadras (2004) based upon the Bloom's (1985) economic analogue for resource limitations hypothesis a negative relationship between the degree of water and N co-limitation (C_{WN}) and the yield gap (defined as the difference between actual and attainable seed yield) . Based on a computer simulation study, Sadras (2004) found that the gap between actual and potential yield was lower when water and N equally co-limited the growth of wheat. These findings were further supported by the field studies in wheat and barley (Cossani *et al.* 2010).

The work so far on water and N co-limitation in cereals has been based on pre-sown fixed amounts of N applications whereas in rainfed environments post-sowing nitrogen management is often adjusted in response to in-season rainfall and is an important tool for risk management and for improved N and water use efficiency. Canola and mustard are also indeterminate crops with extended periods of flowering and pod set where the critical stages of growth may differ from cereals. Therefore the dynamics of water and N uptake may differ fundamentally from those of cereals. Water and N co-limitation have not been examined in canola and mustard. Therefore the aim of this work was to further test the hypothesis of increase in seed yield, NUE and WUE in indeterminate plants (canola and mustard) with variation in water and N co-limitation under post-sowing nitrogen management.

6.3. Materials and Methods

6.3.1. Field experiments and site description

Field experiments were conducted at two sites in South Australia; a medium rainfall site at Roseworthy (latitude 34.53 °S; longitude 138.72 °E), between 2011and 2013, and at a high rainfall site near Tarlee (latitude - 34.15°S; longitude 138.73°E) in 2013. The long term annual average rainfall for Roseworthy is 440mm with the growing season average rainfall (defined in South Australia as rainfall from April to October (French and Schultz

1984)) of 329 mm and the long term annual rainfall of Tarlee (Mid-North High Rainfall Zone) is 527mm with growing season rainfall of 374mm. Three years of this study experienced contrasting amounts and patterns of rainfall (Fig: 1).Soil type at the experimental sites was calacarosol for 2011 and 2012 experiments. In 2013, soil type at Roseworthy was chromosol and vertosol (medium clay over medium-heavy clay) at Tarlee (Isbell 2002) with an alkaline trend down the profile at both sites.

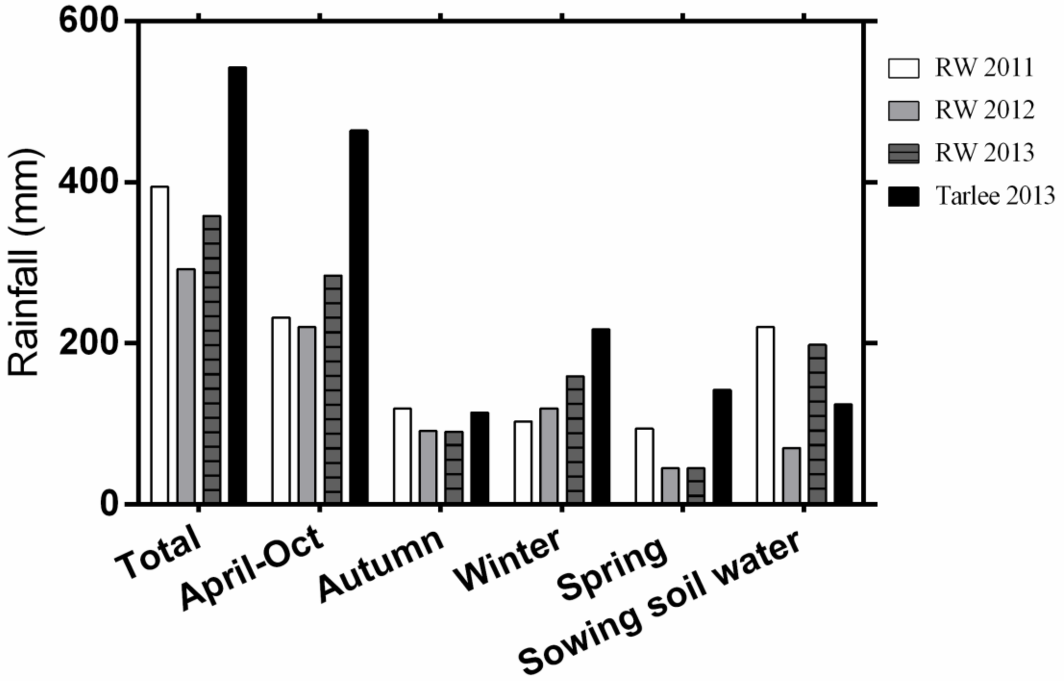

Figure 1: Rainfall received during different seasons of the year between 2011- 2013 for Roseworthy (RW) and Tarlee.

6.3.2. *Cultivars and Nitrogen management*

In the first two years of experiments, two mustard and four canola cultivars (including two triazine tolerant (TT) and two non-TT cultivars) were evaluated under different N application strategies. N treatments comprising three N rates (0, 100 and 200 kg N ha^{-1}) were applied at targeted phenological growth stages. Canola and mustard varieties with similar maturities but with differences in early vigour were selected to represent varieties commonly grown in the region. They included open pollinated (OP), hybrid, conventional, TT and Clearfield varieties (Table: S1). In the third year only a single Clearfield canola variety (Hyola 575CL) was tested under single and split applications of 100 kg N ha^{-1} and 200kg N ha^{-1} of N on medium and high rainfall sites.

Nitrogen treatments were designed to generate a range of biomass and canopy size and targeted at specific growth stages in 2011 and 2012. Two control treatments were used: a nil control with no N and a high N control in which a total of 200kgN ha^{-1} was applied in five equal split applications (rosette (GS30), green-bud (GS51), start of flowering (GS61), start of pod filling (GS67) and 10% pod maturity (GS71)) to maintain a steady supply of N throughout the season. Crop growth stages were recorded by using the BBCH canola scale (Lancashire *et al.* 1991). These controls were designed to provide a boundary function of crop response to N in both years. All other treatments were designed to examine the effects of N supply at a specific growth stage. In 2013, five different N application strategies were used: three N rates (0, 100 and 200 kg N ha^{-1}) (as granular urea) by two timings of either all the N applied just after seedling emergence or equally split among the rosette stage (GS30), green bud appearance (GS51) and at first flower (GS60). In all experiments N was top-dressed by hand as close as possible to the desired growth stage either when the soil was wet or if rainfall was forecast within 24h after fertiliser application. In addition, two water regimes (irrigated and rainfed) were used at Roseworthy in 2013. In the irrigated plots, drip irrigation used at the rosette stage (GS31) to wet the soil profile to the drained upper limit (DUL) to100cm depth, which was equivalent to 60 mm of irrigation. Treatments were replicated six times at Tarlee and three times at Roseworthy in all experiments. The experiments of 2011 and 2012 were arranged in split plot design with cultivars as main plot treatments and N management strategies as subplot treatments. In 2013, a Factorial + added control analysis of variance was used.

6.3.3. *Crop management*

The trials were sown with a cone seeder with knife point drill and press wheels at a depth of 15mm. Plots were 10 metres long comprising 6 rows with 250 mm inter-row width. Basal fertilisers were 10 kg N ha^{-1} and 11 kg P ha^{-1} as diammonium phosphate (DAP) applied at sowing and 100 kg S ha^{-1} as a pre-plant gypsum application. Seeding rates were adjusted on the basis of a germination test to achieve a plant establishment of 35 plants m^{-2}. Weeds were controlled by a pre-sowing application of glyphosate (2.8 L ha^{-1}) and by hand weeding in the crop when required. To avoid any early damage by insects, chloropyrifos (Lorsban 700 ml ha^{-1}) was sprayed two days after sowing. Slugs and mice were managed by applying snail bait (5 kg ha^{-1}) and bromadiolone (Mouse off @ 2-4 kg ha^{-1}) when necessary. Overall weed and disease incidence was minimal at both sites.

6.3.4. *Measurements and sampling*

At maturity each plot was harvested with a small plot harvester after the ends were trimmed and seed yield was estimated based upon the seed yield of experimental plots.

Soil moisture content to a depth of 100 cm was measured pre-sowing and at maturity by using a 4cm diameter hydraulic core. The change is soil water over 0-100cm was used to estimate crop water use (CWU) assuming no drainage below the root zone:

$$\text{CWU} = \text{Growing season rainfall} - (\text{Soil moisture at harvest} - \text{soil water at sowing}) \qquad (1)$$

Water use efficiency (WUE) was calculated as the ratio between yield and water used from sowing to harvest. Soil N to a depth of 100 cm was measured for total N content present in soil (Ammonium N + Nitrate N) by chemical analysis. To measure shoot N uptake, four plants were randomly taken from each plot at maturity (GS99), and dried in an oven at 80 °C for 48 hours. The samples were ground to pass a 2mm sieve and the N concentration of the whole plant was determined with a LECO combustion analyser. Nitrogen concentration in seed was measured by using the near infra-red grain analyser (Cropscan 1000-B). The efficiency of N for canola was calculated by using the following formulae (Fageria and Baligar 2005; Rathke *et al.* 2006):

$$\text{N use efficiency for seed yield (NUE}_{\text{GY}}\text{) (kg kg}^{-1}\text{)} = \frac{\text{Seed yield}}{\text{N supply (Fertiliser+Soil}} \quad (2)$$

6.3.5. Variables used in assessments of yield gaps, different stress and co-limitation indices

Several restrictions were imposed on the calculations and analysis of results reported in this study. Cossani *et al.* (2010) highlighted the role of the independent variable in the calculation of stress and co-limitation indices. They found that changing of the variable values in calculation of stress and co-limitation indices functions did not modify the conclusions. However, calculation of variables from independent source is still important for avoiding circularity in the data. In the study reported here, we calculated or adapted the variables from independent sources. Firstly, simulated maximum attainable yield (Ya) was calculated for each species and location rather than a single value for the region (Table: S2). Maximum attainable yield (Ya) of 4.2 t ha^{-1} for canola and 4.3 t ha^{-1} for mustard was used to calculate the yield gaps for Roseworthy, whereas Ya of 5.1 t ha^{-1} for canola was used for Tarlee (Table: S2). To calculate the WSI, a fixed evaporation of 120mm and a water use efficiency of 15 kg ha^{-1} mm^{-1} were used (Robertson and Kirkegaard 2006). As argued earlier, simulated soil evaporation and WUE (Table: 2) were not used in order to avoid circularity in estimates of parameters data as simulated

Ya was used in the calculation of WSI. A similar approach was used for the calculation of NSI. Maximum N requirement per ton of seed yield was calculated by using the simulated maximum N concentration in seeds (4.5% for canola and 4.49% for mustard) of Ya, and a recent reported NHI of 0.66 for Mediterranean environments was used (Papantoniou *et al.* 2013).

6.3.6. Assessments of yield gaps, N and water stress levels

The **A**gricultural **P**roduction **S**ystems s**IM**ulator (APSIM) was used to simulate the water limited maximum attainable yield (Ya) of canola and mustard between 2001-2013 for Roseworthy and Tarlee. This model has been validated and used for canola in medium and high rainfall areas of Australia (Robertson *et al.* 2002b; Robertson and Kirkegaard 2006). Soils similar to the experimental sites were selected from Australian soil resource information system for the simulations; for Roseworthy Redbanks Site No. 259 and Tarlee Site No. 279 for Tarlee was used in simulation studies. The water limited attainable yield for canola and mustard was estimated from the simulation study from actual rainfall by making N a non-limiting factor. Model default values were used for all remaining parameters. The yield gap was calculated as the difference between the observed yields and the maximum attainable yield (Sadras 2004; Cossani *et al.* 2010).

Water stress index (WSI) (Eq.3) was calculated as the ratio between water requirement for maximum attainable yield and actual water use (Cossani *et al.* 2010). To calculate the water requirements for maximum attainable yield, yield was calculated by using the maximum attainable WUE (15 kg $ha^{-1}mm^{-1}$) for canola and a soil evaporation of 120 mm for this environment (Robertson and Kirkegaard 2006) .

$$\mathrm{WSI} = 1\text{-}\left(\frac{\text{Actual water used (mm)}}{\mathrm{Y_a\ t\ ha^{-1}} \times (0.015\ \mathrm{t\ ha^{-1}\ mm^{-1}})^{-1} + 120\mathrm{mm}}\right) \qquad (3)$$

Nitrogen stress index (*NSI*) (Eq.4) was calculated as the ratio between N requirement for maximum attainable yield and the observed N uptake. Calculation of NSI involved estimation of N requirement to achieve the maximum attainable yield. It was calculated with a reference requirement of 68 kg N ha^{-1} t^{-1}, which is equivalent to 4.5% N in seed and nitrogen harvest index (NHI) of 0.66 (Papantoniou *et al.* 2013) .

$$\mathrm{NSI} = 1\text{-}\left(\frac{\text{Actual N uptake (kg N ha}^{-1}\text{)}}{\mathrm{Y_a}\ \text{t ha}^{-1}\ \times 68\ \text{(kg N t}^{-1}\text{)}}\right) \tag{4}$$

Stresses due to limitation of available resources over the full growing season were taken into consideration for this study (Cossani *et al.* 2010) instead for the critical period of yield determination (Sadras and Roget 2004).*WSI* and *NSI* ranged from 0 (no stress) to 1 (maximum stress) were used to estimate the total stress from water and nitrogen (T_{WN}; Eq.5), maximum of the two stresses (M_{WN}; Eq.6), co-limitation between water and N stress (C_{WN}; Eq 7) and the degree of co-limitation based on total stress(CT_{WN}; Eq.8) and maximum stress(CM_{WN}; Eq.9) were calculated.

$$\mathrm{T_{WN}} = \mathrm{NSI} + \mathrm{WSI} \tag{5}$$

$$\mathrm{M_{WN}} = \mathrm{Max}\ (\mathrm{NSI}, \mathrm{WSI}) \tag{6}$$

$$\mathrm{C_{WN}} = 1\text{-}|\mathrm{NSI}\text{-}\mathrm{WSI}| \tag{7}$$

$$\mathrm{CT_{WN}} = \mathrm{C_{WN}}\ \mathrm{T_{WN}}^{-1} \tag{8}$$

$$\mathrm{CM_{WN}} = \mathrm{C_{WN}}\ \mathrm{M_{WN}}^{-1} \tag{9}$$

Regression analysis on this data set was used to explore the relationships of yield gap, WUE, NUE_{SY} for different stress index for canola and mustard under different nitrogen management strategies and water regimes. The analysis also assumes that there is no other major limiting factor that may interfere with water of N responses

6.4. Results

At Roseworthy, growing season rainfall was similar in 2011 and 2012 (232 mm in 2011 and 220 mm in 2012) but considerably higher in 2013 (284 mm). Soil moisture at sowing was 220mm, 70mm and 198mm for 2011, 2012 and 2013 respectively. Growing season rainfall was 488mm at Tarlee and sowing soil moisture was124mm in 2013. At Tarlee, starting soil moisture was 124mm with the growing season rainfall of 423mm. These different water regimes imposed quite different water stress, which was reflected in *WSI* (range 0.05 to 0.6). At Roseworthy, the highest *WSI* was found in 2012 (range: 0.045-0.61) and the lowest in 2011 (range: 0.02-0.16). In 2013, *WSI* for different N treatments was 0.22-0.44, whereas the range was much smaller at Tarlee (range: 0.32-0.36). In contrast, crops remained at high stress level for N in all experiments (range 0.64 to 0.97) and these were consistently higher than the WSI (Fig: 2). There was no difference between canola and mustard in this respect. At Roseworthy, the highest *NSI* was observed during 2012 (range: 0.82-0.97) followed by 2011 (range: 0.69 - 0.97) and 2013 (range: 0.64 - 0.86), respectively. The range of *NSI* at Tarlee (0.73- 0.79) was smaller than Roseworthy.

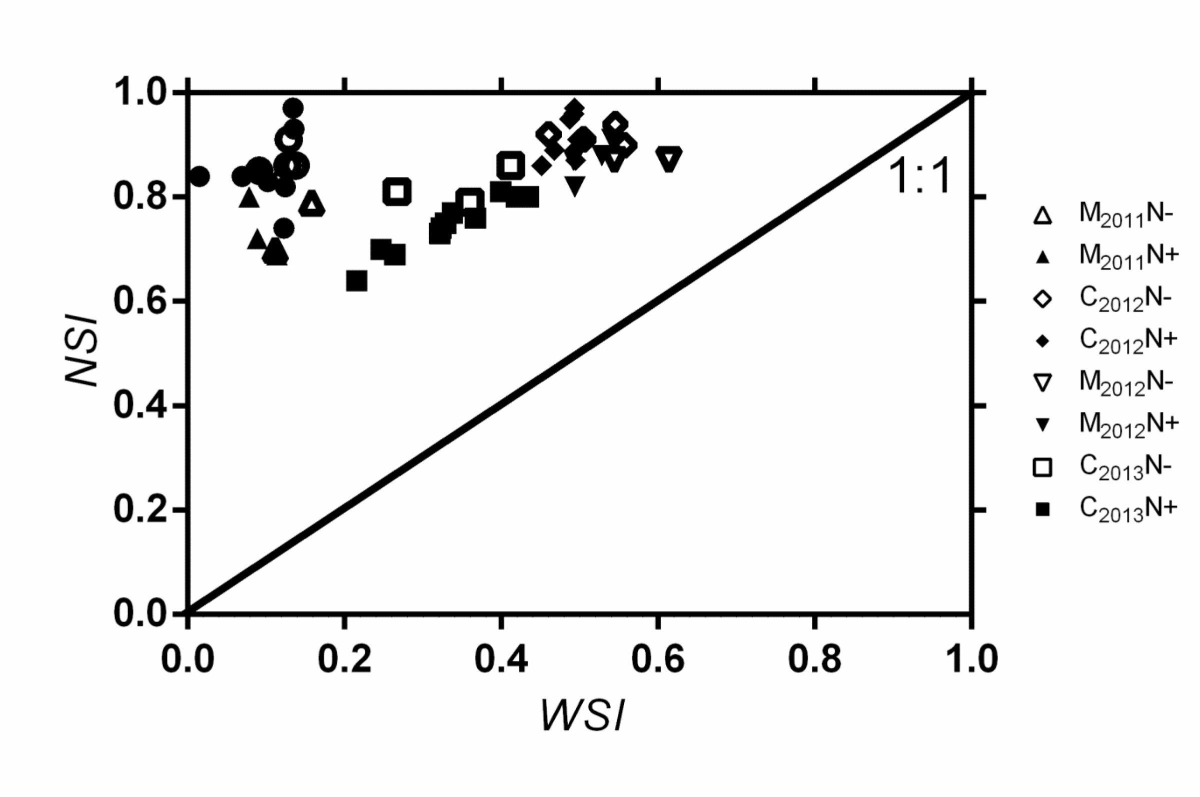

Figure 2: Water stress index (WSI) *v.* Nitrogen stress index for canola and mustard between 2011-2013 at Roseworthy and Tarlee.

Yield gaps ranged from -1.1 to -3.8 t ha^{-1} across the different treatments and growing seasons. The highest yield gap of -3.8 t ha^{-1} was observed in mustard during 2012 whereas the smallest yield gap (-1.1 t ha^{-1}) was observed in canola during 2011 (Table 1). Yield gaps were reduced with the application of N in all treatments across all growing seasons for all the canola and mustard cultivars, without any clear effect of timing and rate of N application (Table 1).

Table 1: Yield gaps (defined as the difference between the maximum attainable yield andactual yield) for canola and mustard between 2011 and 2013 at Roseworthy and in 2013 at Tarlee In 2013 crops at Roseworthy were grown as rainfed (RF) or irrigated (I) crops

	Yield gaps (t/ha)							
2011	All		N0		N100		N200	
	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Canola	-3.1	-1.1	-3.1	-2.3	-2.3	-1.6	-2.2	-1.1
Mustard	-2.8	-1.8	-2.8	-2.7	-2.2	-1.9	-1.9	-1.8
2012								
Canola	-3.6	-2.7	-3.6	-3.1	-3.4	-2.8	-3.2	-2.7
Mustard	-3.8	-3.3	-3.8	-3.8	-3.4	-3.3	-3.3	-3.3
2013								
Roseworthy RF	-2.5	-1.1	-2.5	-2.5	-1.6	-1.4	-1.3	-1.1
Roseworthy I	-2.9	-2.1	-2.9	-2.9	-2.46	-2.46	-2.3	-2.1
Tarlee	-2.6	-2.1	-2.6	-2.6	-2.4	-2.1	-2.2	-2.1

Yield gaps were negatively related to both T_{WN} and M_{WN} stress indices (Fig: 3a, b) and were smaller at the higher values of water-N co-limitation, either expressed by CT_{WN} or CM_{WN} (Fig 3 c, d). Seasonal variation in the reduction of yield gap with the degree of co-limitation was observed for both CT_{WN} and CM_{WN}. Yield gap reduction was more sensitive to the degree of co-limitation during 2012 and 2013 than 2011 (slope for 2012 and 2013: 9.04 t ha^{-1} per unit of CT_{WN} v 2.96 t ha^{-1} per unit of CT_{WN} in 2011) (Fig: 3c).

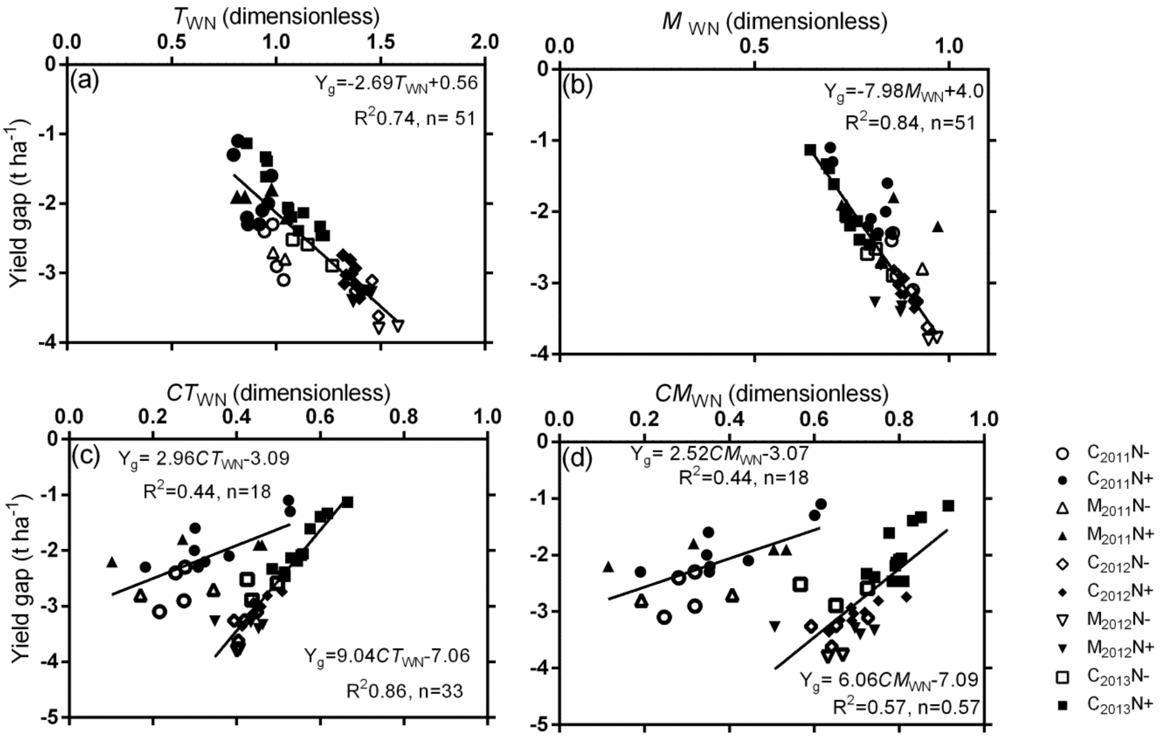

Figure 3: Relationship between yield gap (Yg t ha^{-1}) and various indices for stress and co-limitation: (a) total water-N stress index (T_{WN}); (b) maximum water-N stress index (M_{WN}); (c) degree of co-limitation for intensity of total stress (CT_{WN}); and (d) degree of co-limitation for the intensity of maximum stress (CM_{WN}) for canola and mustard between 2011-2013

Similar trends were also found in CM_{WN} but the magnitude of yield gap reduction was lower than for CT_{WN} (Fig: 3d), as co-limitation based on total stress was always lower than co-limitation for maximum stress from the single most limiting factor, either N or water. Similar to the yield gap, T_{WN} and M_{WN} were negatively related to WUE (Fig: 4a, b) but there was a seasonal difference in response to T_{WN}. Water use efficiency was higher and decreased sharply with the increase in total stress during the seasons with high *WSI*. There was no seasonal variation in the relationship between WUE and CT_{WN} or CM_{WN} (Fig: 4c, d) though improvement in WUE was almost double in CT_{WN} than CM_{WN} (slopes: 11.66 mm kg^{-1} ha^{-1} per unit of CT_{WN} *v*. 5.84 mm kg^{-1} ha^{-1} per unit of CM_{WN}). No relationship was found between NUE_{GY} of canola and mustard and any of the stress indices (Fig 5a, b, c, d).

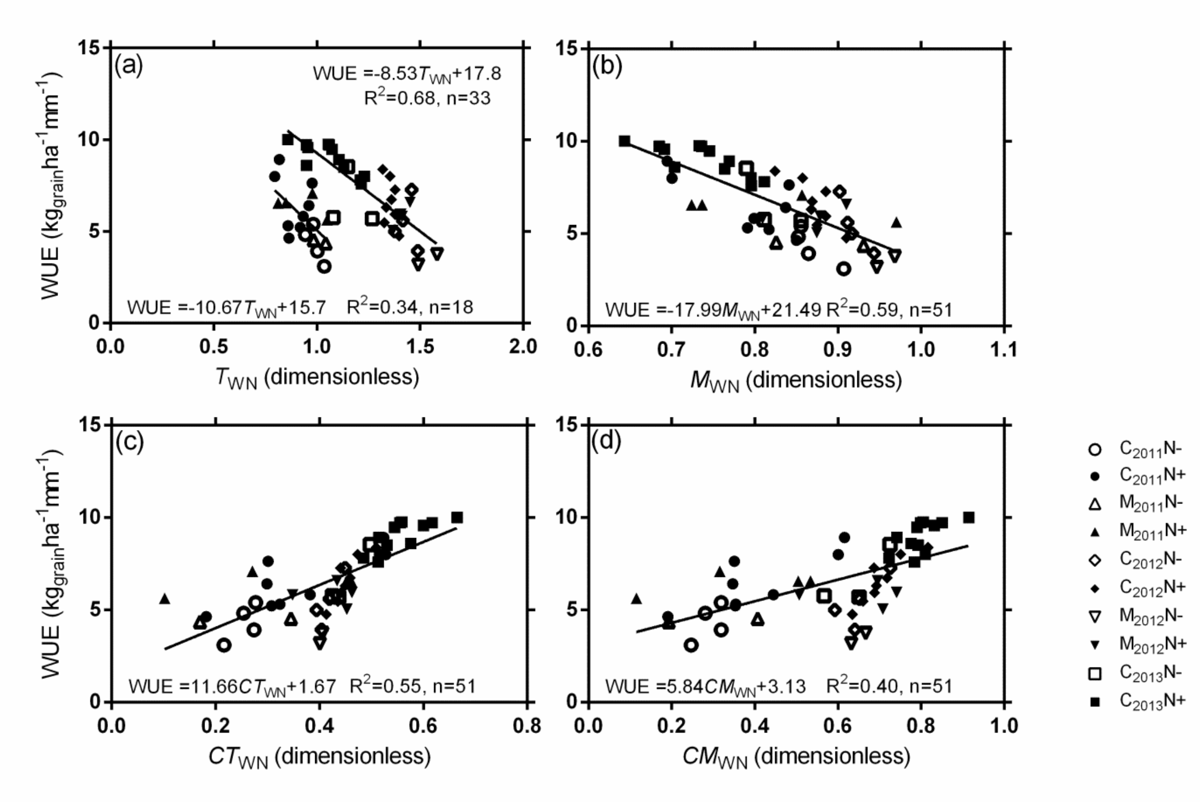

Figure 4: Relationship between water use efficiency (WUE kg ha^{-1} mm^{-1}) and various indices for stress and co-limitation: (a) total water-N stress index (T_{WN}); (b) maximum water-N stress index (M_{WN}); (c) degree of co-limitation for intensity of total stress (CT_{WN}); and (d) degree of co-limitation for the intensity of maximum stress (CM_{WN}) for canola and mustard between 2011-2013.

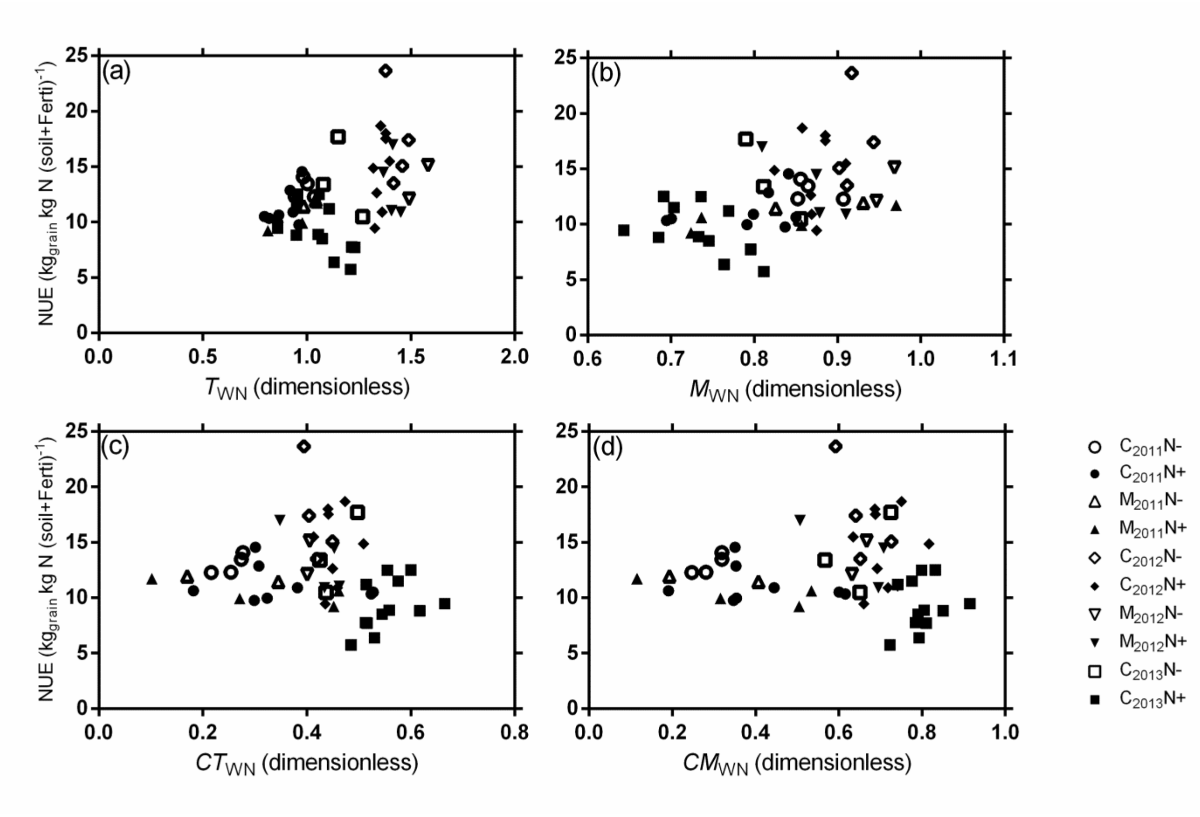

Figure 5: Relationship between nitrogen use efficiency for seed yield (NUE_{SY} kg kg^{-1}) and various indices for stress and co-limitation: (a) total water-N stress index (T_{WN}); (b) maximum water-N stress index (M_{WN}); (c) degree of co-limitation for intensity of total stress (CT_{WN}); and (d) degree of co-limitation for the intensity of maximum stress (CM_{WN}) for canola and mustard between 2011-2013.

6.5. Discussion

Previous approaches with data from simulation models (Sadras 2004; Sadras and Roget 2004; Sadras 2005) or combined modelled and experimental data (Sadras *et al.* 2004), or experimental data (Cossani *et al.* 2010) of cereals suggested a positive relationship between the yield and co-limitation of N and water. Results of this study for canola and mustard are in accord with those reported by Sadras (2005) and Cossani *et al.* (2010) for cereals. Yield gap was reduced and WUE increased by an increased degree of co-limitation. However, NUE_{SY} was not significantly related to the degree of water-N co-limitation. The maximum yield gap for canola (-3.6 t ha^{-1}) and mustard (-3.8 t ha^{-1}) occurred during the dry year of 2012, which is ~1 t ha^{-1} higher than the largest yield gap reported for wheat (-2.7 t ha^{-1}) in low rainfall Mallee region of Australia (Sadras 2004). These results indicate that canola and mustard may be more sensitive than wheat in medium rainfall areas during dry conditions. Interestingly, yield gaps found to be more strongly correlated with *NSI* than *WSI* (Fig: 6). However, crop response to N can be limited not just by N supply but also by the stress imposed by water availability as N uptake is a function of plant available water (Campbell *et al.* 2004). It seems that *WSI* is associated with the distribution of rainfall and not just total rainfall as lower rainfall in spring (September- November) resulted in higher *WSI*.

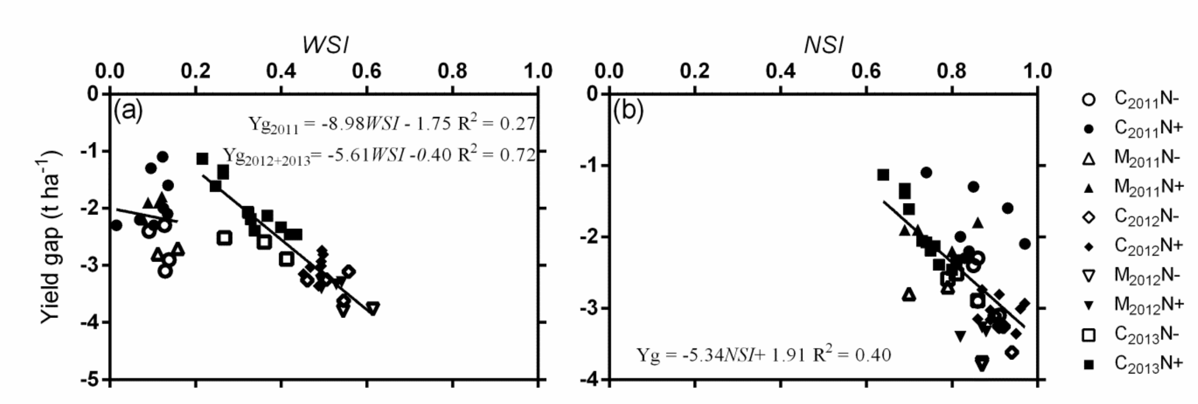

Figure 6: Relationship between yield gap (Yg t ha^{-1}) and indices for stress: (a) water stress index (*WSI*); (b) nitrogen stress index (*NSI*)

On the other hand, *NSI* was inversely proportion to the growing season rainfall; higher growing season rainfall resulted in lower *NSI*. The results reported here also provide some explanation for the poor response to N application observed in canola and mustard. The average yield gap reduction with N in this study was 1.0 t ha^{-1} (range: 0.5 to 2 t ha^{-1}). Sadras and Roget (2004) also reported average N response of 1.1 t ha^{-1} in wheat in low rainfall conditions of the Mallee region of Australia. All the N treatments (rate + timing) in this study did not substantially alter the relationship of yield gaps and WUE with different stress and co-limitation indices as most of the N treatments appeared on same line (Fig: 3 & Fig: 4).

In the study reported here, T_{WN} and CT_{WN} represent the additive effect of water and N on stress and co-limitation whereas M_{WN} and CM_{WN} indicate the effect of the most limiting factor of these two resources.Sinclair and Park (1993) found that water and N both limit the yield of most rainfed crops in semiarid regions and this argument seems to hold true for canola and mustard in this Mediterranean environment. Both T_{WN} and M_{WN} stress indices were negatively related with the yield gap. However, the stress imposed by single limiting factor (M_{WN}) can be more limiting, once it reaches above a certain level (eg: $M_{\mathrm{WN}} = 0.50$ for yield (Fig: 3b), and $M_{\mathrm{WN}} = 1.19$ for WUE (Fig: 4b). Further analysis of M_{WN} revealed that *NSI* was the M_{WN} in most situations (Fig: 7b) and was therefore a more important yield limiting factor than water (Fig: 7a, b).

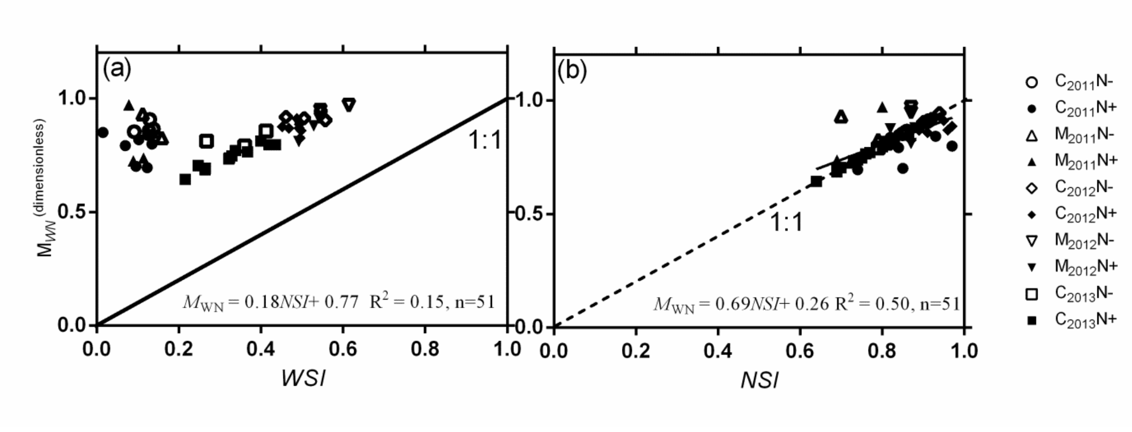

Figure 7: Maximum water N stress index M_{WN} *v.* (a) *WSI*; and (b) *NSI* for the data set used in the analysis.

It could be argued that reported comparison of *WSI* with yield gap was done on the water limited maximum attainable yield for the region. To rule out this we compared simulated water limited maximum attainable yield with simulated yield under non-limited water conditions (Fig: 8). Water limited yield was estimated with actual growing season rainfall whereas yield for non-limited water conditions was simulated by making water unlimited within APSIM. In both scenarios N was made unlimited and all other default parameters were used.

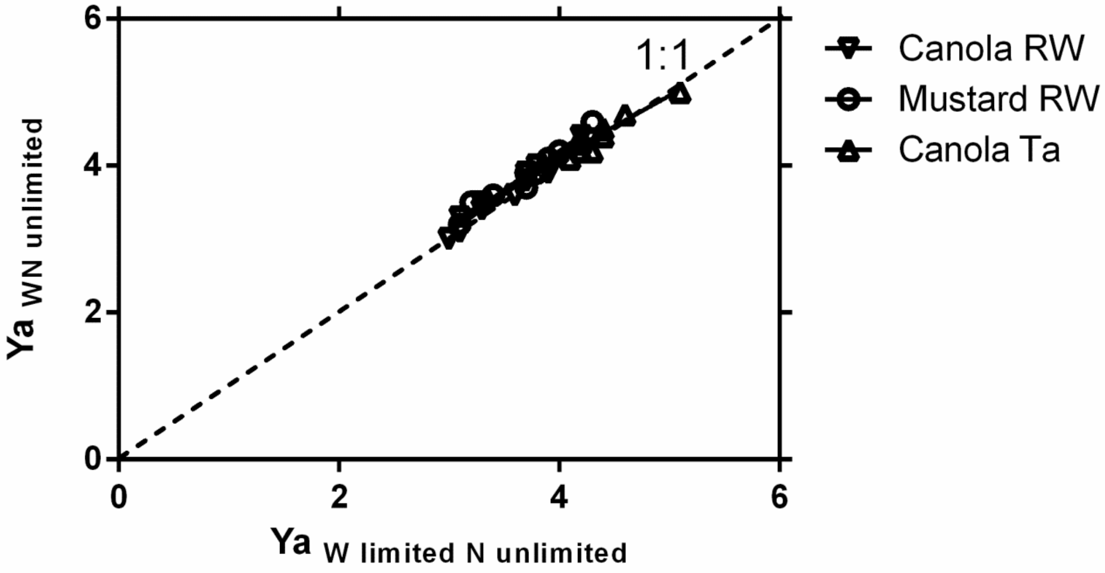

Figure 8: The relationship between the simulated maximum attainable yield under non-water limited conditions (Ya $_{\text{WN unlimited}}$) and Maximum attainable yield under water limited conditions calculated based upon actual rainfall received (Ya $_{\text{W limited and N unlimited}}$) between 2001-2013 for Roseworthy and Tarlee.

Interestingly, it was revealed that water limited and water unlimited maximum attainable yield very similar and close to the 1:1 line, which suggests that the yield of canola and mustard was rarely limited by water at Roseworthy and Tarlee. Robertson and Kirkegaard (2006) also reported that seed yield of canola is not limited by water stress when seasonal water supply is greater than 450mm. Steps were taken to avoid the circularity of data in the calculation of the WSI (eq.3) and NSI (eq.4); the numerator was derived from experimental data whereas the values for the denominator were obtained from independent sources (simulated, published literature and calculated). Despite this, it was still not practical to compare the NSI with NUE_{GY} or WSI with WU or WUE due to same parameters in numerator and denominator.

NSI is estimated on the basis of seed N content and represents the ratio between seed N uptake and N uptake required to achieve maximum attainable yield. Furthermore, it can be argued that the main reason for low N uptake in seed in our study was the lower translocation of N from the shoot to seed. The rate of N translocation from the shoot to seed (NHI) for our study was much lower (0.30 and 0.40, data not shown here) than 0.80-0.90, reported by Hocking and Stapper (2001) and 0.66 reported by Papantoniou *et al.* (2013). Low NHI in these conditions were mainly related to post-flowering water stress.

The analysis of the present study is in line with the previous reports of co-limitation of water, which showed that under rainfed conditions N availability needs to match the availability of water for minimising the yield gap. These findings support the Liebscher’s law of the optimum as proposed for resources in agriculture by De Wit (1992). De Wit proposed that resources get used more efficiently with increasing availability of other resources. Previous studies provided support for this law in cereals with water x N co-limitation. This study provides the first empirical evidence of N-water co-limitation in an indeterminate crop (canola and mustard) with different post-sowing N management strategies under different water regimes. Adjusting N inputs to water availability leads to an increase in the degree of co-limitation in water and N, which increase yield and reduce the gap between actual and attainable yield. This improvement in yield with greater N-water co-limitation is probably due to avoidance of excessive crop water use during the early stage of crop development (Norton and Wachsmann 2006), which could improve crop growth during later critical stages. The analysis showed that in our study environment, nitrogen was a bigger limiting factor to yield than water, which indicates a mismatch between the demand and supply of N. However, the yield gap reduction with the application of higher rates of N at five key growth stages was not substantially huge due to limited N uptake under water stress. *WSI* was more closely related to the rainfall distribution than the total rainfall over the growing season rainfall whereas the *NSI* was related to the growing season rainfall indicated the continuous demand of N throughout the growing period for these crops. However, the yield gaps were not zero even in the high N control, where N was supplied at five key growth stages. It indicated the N stress over the growing period is related to poor poor physiological efficiency during post-flowering period which reflected in low NHI, might be related with water stress due to low rainfall in spring in this Mediterranean environment. Future studies need to be more focused on the interaction of pre and post-flowering WSI and NSI in devising management tools for canola and mustard in this Mediterranean environment.

6.6. Conclusions

The study provides the first empirical evidence that yield of canola and mustard is co-limited by water and nitrogen under the post-sowing nitrogen application in the Mediterranean environment of South Australia. Yield gaps of both crop species were more strongly related with the *NSI* than *WSI*. *WSI* and NSI were negatively associated with the spring rainfall and the growing season rainfall, respectively. Both T_{WN} and M_{WN} stress indices were also negatively related with the yield gap and WUE. Yield gaps reduced with the increased degree of water-N co-limitation, either expressed by CT_{WN} or CM_{WN}. However, there was some seasonal variation in the reduction of yield gap with the degree of co-limitation as CT_{WN} and CM_{WN}. No relationship was found between NUE_{SY} of canola and mustard and any of the stress and co-limitation indices. The present study also indicated that stress imposed by a single limiting factor (M_{WN}) can be more limiting, once stress from single factor reaches to a certain level. The analysis showed that N is the bigger limiting factor than water, which may be due to low N uptake efficiency during the pre-flowering period and low physiological efficiency during the post–flowering period. Crop response to N may be reduced by water stress due to low spring rainfall because even the application of the higher rate of N at five key growth stages did not reduce the yield gap. So the future studies need to focus on the interaction of pre- and post-flowering *WSI* and *NSI* in devising management tools to reduce the yield gaps.

6.7. Acknowledgements

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6.8. Supplimentary Tables

Table S1: Details of the cultivars used for experiments during 2011, 2012 and 2013.

Cultivars	Type	Species	Origin	Maturity
AV Garnet	Open pollinated (OP)	Canola	Australia	Mid
FighterTT	Open pollinated (OP)	Canola	Australia	Early- mid early
Hyola555TT	Hybrid	Canola	Australia	Mid-Mid early
Hyola575CL	Hybrid	Canola	Australia	Mid-Mid early
Oasis CL	Open pollinated (OP)	Mustard	Australia	Early
Varuna	Open pollinated (OP)	Mustard	India	Early

Table S2: Range of simulated maximum attainable yield (Ya), soil evaporation (es) and water use efficiency (WUE) for canola and mustard between 2001-2013 at Roseworthy and Tarlee.

		Simulated Ya, es and WUE					
		Ya (t ha^{-1})		Es (mm)		WUE (kg ha^{-1} mm^{-1})	
Roseworthy		Highest	Lowest	Highest	Lowest	Highest	Lowest
	Canola	4.2	3.0	151	123	13.9	9.2
	Mustard	4.3	3.1	153	127	13.5	9.0
Tarlee							
	Canola	5.1	4.1	147	120	21.0	9.4

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Chapter 7

7. General Discussion

Canola is an important part of Australian farming systems due to its high profitability, benefits for weed control and as a disease-break. Improved adaptation of canola through breeding has pushed its production into low rainfall areas in South Australian Mediterranean environments. Herbicide-tolerant canola systems and canola quality *Brassica juncea* provide an attractive proposition for many growers. Though plant breeding has contributed to an improvement in canola yields, there is still a large gap between actual and attainable yields (Lisson *et al.* 2007; Kebede *et al.* 2010). Moreover, canola is now often grown after cereals on soils with low soil N status, which requires large applications of N to achieve high yields in intensified cereal based farming systems. Recovery of N is generally less than 50% in several crops (Fageria and Baligar 2005) and oilseed rape has a lower NUE than many other major crops (Sylvester-Bradley and Kindred 2009). In water-limited environments, growers are often asked to manage fertiliser N in response to water availability and crop demand (Sadras 2004; Potter *et al.* 2009). Most of the time, N rate and the timing of split applications of N are selected arbitrarily. The physiological investigation of Barłóg and Grzebisz (2004a) shows that there are critical stages in canola growth when high N nutrition is required for better yield formation. In rainfed systems water and N can interact strongly to determine yield and several studies have shown the effects of water availability on N response of crops and *vice-versa* (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012). The concept of co-limitation as a means of understanding the water x N interactions within rainfed farming systems has been studied in cereals. However, to date little is known about the role of N on different growth stages of canola under water limited conditions

(Richards and Thurling 1978; Lewis and Thurling 1994; Norton and Wachsmann 2006). Furthermore, the question of whether the timing of N affects the degree of N- water co-limitation in canola and mustard has not been addressed. Therefore, it was timely to undertake this study on N management for canola in water–limited environments.

The results of this study clearly showed that total dry matter at maturity was the main determinant of canola and mustard seed yield without any considerable improvement in HI (Chapter 3 and Chapter 5). Independence of yield from HI in canola observed in this study was also reported for different B*rassica* species by Lewis and Thurling (1994). In some areas canola can experience greater reduction in biomass production during spring than mustard, due to leaf shedding during flowering, pod and seed fill and in such situations mustard can out yield canola (Hocking *et al.* 1997f). However, there was no dry matter reduction observed in spring in this study (Chapter: 3). Therefore canola and mustard produced similar seed yield during a wet season (2011) whereas seed yield of mustard was 21% lower than canola during a dry season (2012). The lower HI of mustard (0.23 - 0.27) than canola (0.27 – 0.34) was the likely cause of lower seed yield of mustard. Mustard showed superior ability for developing a bigger sink than canola due to earlier and longer flowering even under dry conditions but it was unable to convert that sink into superior seed yield in a dry season. There was very little increase in crop dry matter after flowering in a dry season, which has prevented conversion of extra sink in mustard to seed yield.

Within canola, the yield penalty of TT cultivars as compared to non-TT canola varies with environmental conditions (Beversdorf *et al.* 1988; Robertson *et al.* 2002b). Usually, yield differences among TT and other cultivars tend to be small in low rainfall areas but can be quite large in high rainfall areas (Robertson *et al.* 2002b). Data from national variety testing trials (NVT) for medium (annual rainfall <450mm) and high rainfall sites (annual rainfall >450mm) showed that the average yield penalty in TT cultivars relative to imidazolinone-tolerant(Clearfield) cultivars was 13% in NSW, 12% in SA, 4% in Victoria and 16% in Western Australia (Chapter 2). In this study, the yield penalty in TT cultivars was higher than these state averages and it was also lower in the drier season (2012) than in wet season (2011). On average, TT cultivars produced 35% less seed yield than non-TT cultivars during wet season (2011) and the seed yield penalty was 22% during the drier season (2012). In both years TT cultivars produced fewer seeds m^{-2} than non-TT cultivars but with similar seed weight. The results indicated that the yield penalty of the TT trait is associated with the development of a smaller source and sink capacity as the HI of TT and non-TT cultivars was similar (In 20110.27 *v.* 0.26 and 0.34 *v* 0.34 in 2012.).

Apart from TT and non-TT cultivars another options to choose right cultivars is hybrid or open-pollinated cultivars (OP).A major trend in canola production in Australian has been the widespread adoption of hybrid varieties as seed companies have moved away from releasing OP varieties. Mahli *et al.* (2007) found that hybrid cultivars produced more biomass than OP cultivars and this was reflected in their higher seed yield. In this study in SA, hybrid canola also produced more biomass than OP cultivars but with lower HI, which resulted in similar seed yields in 2011. However in the drier year 2012, hybrid canola produced 34% more biomass and 28% higher seed yield (P=0.007) than the OP cultivars. These results suggest that in environments with high winter rainfall, hybrids are a better option over OP cultivars due to their higher biomass and seed yield production. Seed yield of mustard appears to rely more heavily on post-flowering growth than canola in which yield was more strongly influenced by vegetative growth up to flowering. Early (Varuna) and mid-season (Oasis) maturity of the mustard cultivars used in this study would make them more sensitive to water stress during post-flowering period than canola. HI remained relatively stable across the N treatments. Independence of seed yield from HI in canola and mustard observed in this study was also reported for different *brassica* species by Lewis and Thurling (1994).

On average, N improved seed yield by 38%, 30% and 42% in 2011, 2012 and 2013 respectively under rainfed conditions at Roseworthy,. However, when supplementary irrigation was applied at the rosette stage, N improved seed yield by 69% in 2013. At Tarlee, seed yield was only improved by 16% with applied N under rainfed conditions. The lower crop response to N at Tarlee was associated with higher soil N at sowing (142 kg N ha^{-1}) at this site compared to Roseworthy. The seasonal differences in crop responsiveness to applied N may be related to different water regimes, which can affect N uptake and utilisation (Sadras 2004; Sinclair and Rufty 2012). Apart from the influence of water availability, there was variation in the response to applied N among different cultivars of canola and mustard (Fig: 1). Yau and Thurling (1987) reported significant difference in N utilization in Australian spring cultivars of canola. Svečnjak and Rengel (2006) and Balint *et al.* (2008) also reported that the extent of response to N can vary among cultivars. In dry conditions, mustard cultivars were more responsive to N than any canola cultivars but in a wet season hybrid canola cultivar Hyola575CL was more responsive to applied N than other canola and mustard cultivars. The regression lines for dry and wet years differed significantly from each other, which showed that N response was greatly influenced by the seasonal water availability. Interestingly, this analysis showed that in a dry year if soil N supply is adequate for 1.4 t ha^{-1} seed yield, then the application of fertiliser N is unlikely to increase seed yield.

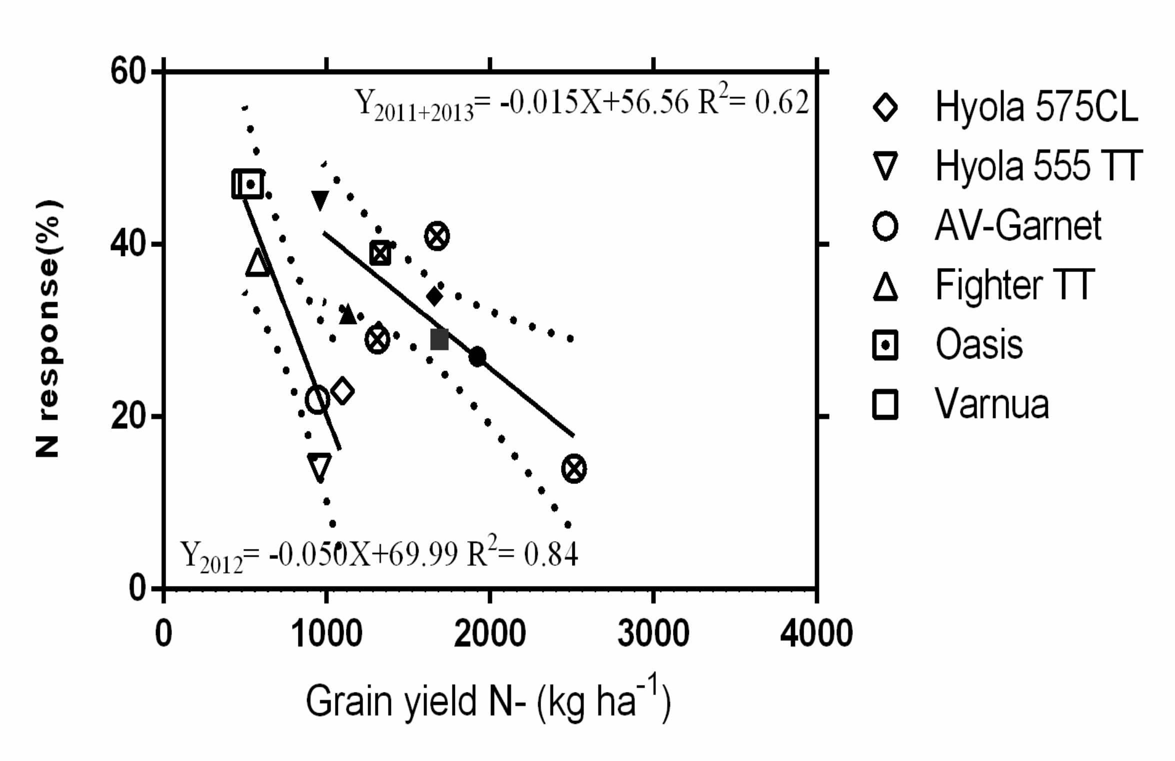

Figure 1: Percentage increase in seed yield with N fertiliser over control (N0) under different water regimes between 2011 and 2013. Solid points represent the data from 2011, hollow points for 2012 and marked points for 2013. Solid lines represent the linear regression and dotted points represent the 95% confidence intervals

Cultivars used in the experiments were more responsive to an early N application at the rosette stage than later at green-bud or at flowering. Canola and mustard achieved 85% and 94% of the yield of the non-limiting N treatment when N was applied at rosette stage in 2011 and 2012, respectively. Further delaying N application after the rosette stage resulted in a larger yield penalty. The importance of the period from rosette to green-bud stage for N application was also identified in previous studies by assessing plant N status at various growth stages (Bernardi and Banks 1993; Hocking *et al.* 1997b). However in 2013 experiments at two different sites under three different water regimes, found that the timing of N application (single vs split) of 100 kg N ha^{-1} and 200 kg N ha^{-1} had no effect on seed yield of canola. The rate or timing of N applications had little effect on total water use of canola and mustard cultivars in the wetter seasons at Roseworthy (2011& 2013). These results differ from the findings of Norton and Wachsmann (2006) who found that water use by canola and mustard cultivars was influenced by N rate in the Victorian Wimmera. However, their findings are in line with the results from the 2012 growing season at Roseworthy and the experiment at Tarlee in 2013, in which water use of canola cultivars was more responsive to N rate than the timing of application, whereas water use of mustard was more responsive to the timing of application in 2012 at Roseworthy. Mustard used less water during the pre-flowering phase than canola, which was attributed to its shorter pre-flowering duration than canola. Therefore, a greater proportion of water use in mustard was associated with dry matter accumulation during the post-flowering period. Thurling (1974) also found that mustard produced 85% of its dry matter during the post-flowering growth whereas canola produced 55% of its total dry matter during this phase.

The values for NHI (0.13 - 0.49) in the experiments reported here are lower than recently reported NHI of 0.61 for canola by Papantoniou *et al.* (2013) but much lower than those reported by Hocking and Stapper (2001). There were also differences in NHI between different growing seasons, with the mean values being higher in 2012 and 2013 compared to 2011. The reason for lower NHI in a higher rainfall spring season than a drier spring could be associated with greater post-flowering N uptake and lesser reliance on remobilisation of N, which was the main reason for high NHI in 2012 and 2013 than 2011 at Roseworthy. The decline in NHI with high N applications in the wetter season indicated inefficient use of applied N fertiliser.

The studies reported in this thesis showed that soil water available for crop use has a large influence on crop water use, N uptake and NUE_{SY} of canola and mustard in this Mediterranean environment. Contrasting water availabilities between growing seasons had a remarkable effect on water and N used by canola and mustard. The interactions between N and crop water use are well known (Sadras 2004; Norton and Wachsmann 2006; Sinclair and Rufty 2012). Nitrogen treatments changed the proportion of crop water use during the pre- and post-flowering phases. On average, pre-flowering water use increased with N application in both years. Higher crop water use during the pre-flowering period in response to N application was associated with water extraction from deeper layers in the soil profile, possibly due to higher root density. Although no root data were collected in these experiments, soil water distribution in the profile at flowering supports this conclusion. Surprisingly, canola and mustard failed to dry the soil profile to levels similar to the pre-sowing soil water content in 2012 (Chapter 4, Figure 5). Even though canola has a deep tap-root, it failed to make use of all plant available water at depths >30 cm. The presence of high EC, boron and high pH in these soils may have created inhospitable sub-soil at the trial sites. The clearest example of water accumulation at depth was found in mustard with no N in 2012. In this case, there was build-up in soil water at 90 cm depth between mid-flowering and maturity. It could be argued that lower shoot biomass of mustard without N was reflected in lower root growth, which limited its ability to use soil water deep in the profile. This accumulation in soil water in deeper layers of soil was not evident in treatments with 100 kg N ha^{-1} or 200 kg N ha^{-1} (Chapter 4, Figure 5 g-i). In 2011, lower water use during the post-flowering period (as a proportion of total water use) with 100 kg N ha^{-1} and 200 kg N ha^{-1} was attributed to improvement in crop vigour and pre-flowering water use by N (Norton and Wachsmann

2006). With the exception of mustard with no N application, all other treatments showed some water extraction down to 90 cm in the soil profile.

Total crop water use was directly proportional to the water availability and it had a large impact on total N uptake by canola and mustard. Nitrogen uptake was higher with high water availability;a positive relationship between N uptake and plant available water has been previously reported in wheat (Campbell *et al.* 2004). The effects of water availability on crop responses to N observed in this study are in agreement with Norton and Wachsmann (2006). They also showed that the small changes in crop water use had a large effect on the improvement in seed yield. Higher N uptake in wet conditions improved the N uptake efficiency, N use efficiency, agronomic efficiency and apparent N recovery than the drier conditions. Improvement in N recovery from 30% to 50% with additional irrigation was reported by Schjoerring *et al.* (1995). Nitrogen recovery with the supplementary irrigation at the rosette stage was improved by 21% over the rainfed treatment at Roseworthy in the 2013 experiment.

In the higher rainfall season of 2011, N uptake by the TT canola was lower than non-TT cultivars even though their crop water use was similar. Post-flowering N uptake was also higher in non-TT cultivars, which could be related to their more vigorous shoot and root growth during early growth as water extraction depth for TT and non-TT cultivars at maturity was similar. A positive relationship between longer and vigorous root growth with higher N uptake has been reported previously (Kamh *et al.* 2005) . The timing of N application at different growth stages did not influence the total N uptake, which was affected by the rate of N supply in both years. Understandably, shoot N uptake was an important determinant of crop productivity rather than the supply of N. Similar findings were reported by Cramer (1990) as cited in (Marquard and Walker 1995).

Nitrogen uptake efficiency of cultivars under different N regimes is an important source of variation in NUE (Grami and LaCroix 1977; Yau and Thurling 1987; Möllers *et al.* 1999; Zhang *et al.* 2010). There were no cultivar × nitrogen interactions in this study for N uptake efficiency and other N use efficiency parameters. Nitrogen uptake efficiency of canola and mustard was similar in wetter conditions but mustard had lower N uptake efficiency than canola in drier conditions. Higher rates of N application and delayed N application beyond the rosette stage decreased the agronomic efficiency of canola and mustard in both years. On average, mustard had a higher agronomic efficiency and apparent recovery than canola in dry conditions. These results are consistent with the study of Hocking and Stapper (2001) but lower than the values reported in other studies by Smith *et al.* (1988) and Hocking *et al.* (1997d). Although physiological efficiency of canola and mustard did not differ between the two seasons with contrasting water availabilities, mustard had a lower NUE_{SY} than canola. Values for physiological efficiency of 27 kg kg^{-1} in 2011 and 39 kg kg^{-1} are similar to those reported by Smith *et al.* (1988) in rapeseed and Anderson and Hoyle (1999) in wheat for these Mediterranean environments. Physiological efficiency was lower with the growing season rainfall less than 250 mm (i,e 2011: 232mm and 2012: 220mm) whereas in 2013 (284 mm) physiological efficiency improved to 65 kg kg^{-1} due to high rainfall in winter and it was further improved to 70 kg kg^{-1} with the supplementary irrigation at the rosette stage. N application at the rosette stage improved physiological efficiency over N applied at the green-bud stage and flowering in the wetter seasons. However, the timing of N had no effect on physiological efficiency in the drier growing season. Improvement in physiological efficiency with early application (at the rosette stage) is in agreement with the studies on wheat; the physiological efficiency of wheat improved with an application of N at GS37 (Flag leaf just visible on main stem) over N application at sowing (Whitfield and Smith 1992; López-Bellido *et al.* 2006). TT cultivars had a lower N uptake efficiency than non-TT cultivars in 2011 (higher rainfall) and lower NUE_{SY} due to similar physiological efficiency whereas in dry conditions (2012) all canola cultivars had similar N uptake and use efficiencies. In general, N uptake efficiency decreased with application of N but was not influenced by N rate whereas NUE_{SY} decreased with increased N rate.

On average, WUE did not differ between the TT and non-TT cultivars and the results were consistent with their known differences in radiation use efficiency and transpiration efficiency (Robertson and Kirkegaard 2006). As cultivars used in this study had similar stable carbon isotope ratios, they were expected to have similar transpiration efficiency under different water availability. So in this study lower radiation use efficiency of TT cultivars than non-TT cultivars was the reason for low water use efficiency of the TT cultivars. Water use efficiency for seed yield values for canola and mustard cultivars reported here are within the range of 3-18 kg ha^{-1} mm^{-1} reported from 42 different case studies simulated by Robertson and Kirkegaard (2006). In 2013, irrigation at the rosette stage improved WU and WUE of canola. The average WUE of the rainfed canola crop was 7.5 kg ha^{-1}mm^{-1} which increased to 8.7 kg ha^{-1}mm^{-1} in the irrigated crop at Roseworthy. The improvement in WUE with supplementary irrigation at rosette stage indicates that WUE can be improved by improving the sub soil water use.

Sadras (2004) explained the degree of N and water co-limitation in wheat and showed that the yield gap between actual and potential yield was lower in rainfed environment when water and N equally co-limited growth of wheat. Previous analysis with data from simulation models (Sadras 2004; Sadras and Roget 2004; Sadras 2005) or combined modelled and experimental data (Sadras *et al.* 2004), or experimental data (Cossani *et al.* 2010) alone showed a positive relationship between the yield and co-limitation of N and water in cereals. Results of this study for canola and mustard are in accord with those reported by Sadras (2005) and Cossani *et al.* (2010) for cereals. The yield gap was reduced and WUE increased with an increased degree of co-limitation, whereas NUE_{SY} was not significantly related to the degree of water-N co-limitation. The maximum yield gap for canola (-3.6 t ha^{-1}) and mustard (-3.8 t ha^{-1}) occurred during the dry year of 2012, which was ~1 t ha^{-1} higher than the highest yield gap reported for wheat (-2.7 t ha^{-1}) in a low rainfall Mallee region of Australia (Sadras 2004). This comparison indicates that canola and mustard could be more sensitive than wheat in medium rainfall areas during dry conditions. Interestingly, yield gaps were more strongly related with *NSI* than *WSI*. However, crop response to N can be affected by the stress imposed by water availability as N uptake is a function of plant available water (Campbell *et al.* 2004). It seems *WSI* is influenced by the distribution of rainfall rather than just the amount of rainfall as lower rainfall in spring (September- November) resulted in higher *WSI*. On the other hand, *NSI* was inversely proportional to the growing season rainfall; higher growing season rainfall resulted in a lower *NSI*. The average yield gap reduction with N of 1.0 t ha^{-1} (range: 0.5 to 2 t ha^{-1}) indicates poor response of canola and mustard to N application. Sadras and Roget (2004) also reported average N response of 1.1 t ha^{-1} in wheat in low rainfall conditions of the Mallee region of Australia. All the N treatments (rate + timing) in this study did not substantially alter the relationship of yield gaps, WUE and NUE with different stress and co-limitation indices as most of the N treatments appeared on same line. This study also indicated that the stress imposed by a single limiting factor (M_{WN}) can be more limiting, once it is above a certain level. Further analysis of M_{WN} revealed that *NSI* was the main contributor to M_{WN} in most situations and was therefore the bigger yield limiting factor than water. Regarding NSI, the major limitation seems to be related with N uptake as this index represents the ratio between N uptake and N uptake required for achieving the maximum attainable yield.

This study provides the first empirical evidence of N-water co-limitation in an indeterminate crop (canola and mustard) with different post-sowing N management strategies under different water regimes. It is worth noting that the yield gaps were not zero even in the high N control, where N was supplied at five key crop growth stages. This indicated that the N stress over the growing period was related to poor N uptake efficiency in early growth period even when water was not limited, and poor physiological efficiency during the post-flowering period might be related to water stress due to low rainfall in spring.

To sum up these findings some of the key conclusions are:

- Seed yield of canola and mustard in this Mediterranean environment was mainly determined by shoot dry matter rather than changes in harvest index. Therefore, early vigour and dry matter production at rosette stage is necessary for achieving higher seed yields in canola.
- Mustard seed yields were lower than canola in a season with a dry finish, which appears to be related to a greater contribution of post-flowering growth to seed yield in mustard.
- Triazine tolerant cultivars showed dry matter and yield penalty and low NUE_{GY} relative to the non-TT canola; however, their inclusion in cropping programs may be justifiable due to weed control benefits.
- Hybrid canola cultivars consistently had a lower HI than OP varieties and therefore need more dry matter to produce similar yields as open pollinated cultivars of canola. Hybrids appear to be less efficient but may still be a suitable option for areas with high winter rainfall.

- Nitrogen improved seed yield of canola and mustard by increasing shoot dry matter production and yield components. Nitrogen at the rosette stage (GS30) is critical for achieving higher yields in these environments because it promoted inflorescence development and improve sink capacity, which was ultimately reflected in greater seed m^{-2}, and higher seed yield.
- Nitrogen application rates (100 kg N ha^{-1} and 200 kg N ha^{-1}) and timing (single and splits) of N had little impact on yield, total dry matter, water use and WUE. This indicated that N application of 100 kg ha^{-1} adequately supplied the crop demand for N.
- Nitrogen applications did not influence the total water use of canola and mustard cultivars but changed the pre- and post-flowering partitioning of total water use.
- Water extraction depth at flowering was increased by the rate of N applications but all cultivars extracted soil water from similar depths at maturity. At Roseworthy, soil water depletion was limited to a soil depth of 90 cm and at Tarlee canola used water down to 70 cm depth but at both the sites subsoil moisture was not used completely.
- Low NUE_{SY} in these environments was mainly related to the limitation of N uptake, and low N uptake efficiency during pre-flowering period, low physiological N efficiency during post-flowering period, which may be related to water stress in spring season with low rainfall.
- The water use efficiency of the cultivars selected for this study remained similar 5.7 kg $ha^{-1}mm^{-1}$during contrasting availabilities of water but there were large differences in total water use between the seasons. N application improved the WUE. There was large variation in WUE between 2011 and 2013 (2.8 kg $ha^{-1}mm^{-1}$ to 10 kg $ha^{-1}mm^{-1}$) at Roseworthy.

- The average WUE of rainfed crop was 7.5 kg ha^{-1}mm^{-1} and the WUE of the irrigated crop was 8.7 kg ha^{-1}mm^{-1} but the additional water from irrigation water was used almost twice as efficiently as the seasonal WUE (13.8 kg ha^{-1}mm^{-1}). Water use efficiency for yield at Tarlee was 9.3 kg ha^{-1}mm^{-1} and this was unaffected by the rate or timing of N. Improvement in WUE with additional water availability indicate that better use of subsoil moisture may be an avenue for further improvements in yield and WUE of canola in this environment.
- This study provides the first empirical evidence that yield of canola and mustard is co-limited by water and nitrogen under the post-sowing nitrogen application. Yield gaps were more strongly related with the *NSI* than *WSI. WSI* was found to be negatively associated with the spring rainfall whereas *NSI* was inversely related to the growing season rainfall.
- The analysis showed that N is a bigger limiting factor for yield than water, which may be due to low N uptake efficiency during the pre-flowering period and low physiological efficiency during the post –flowering period. Crop response to N may also be reduced by high water stress due to low spring rainfall because even increasing the rate of N did not reduce the yield gap.

7.1. Recommendations for Future research

Based on the findings of this thesis, the following areas for further research are recommended:

- Nitrogen application at the rosette stage (GS30) was critical for achieving higher yields in these environments as there was yield penalty associated with delaying N application beyond the rosette stage. However, greater early vigour and dry matter production could increase water use in the pre-flowering period which could lead to crop water stress during the post-flowering period that can hinder N uptake and translocation and consequently lower yield. So future studies need to quantify how much early vigour is optimum for achieving the potential seed yield of canola in these environments with variable amount and distribution of rainfall.

- Sowing canola in May-June did not reveal any advantage of early sowing in mid-maturity type cultivars. However, many growers are now sowing canola in April. Therefore, it would be interesting to investigate the performance of late maturing cultivars in early sowing in April. Early sowing of late maturing cultivars can provide a longer post-flowering period for sink development, N translocation and can improve the NHI in growing season with rainfall >300mm.
- In two years of study with different cultivars, mustard did not out-yield canola and the HI of mustard remained lower than canola. These differences in HI may be due to less breeding effort so far into mustard. There is a need for breeding effort to develop mustard as a viable crop for medium and low rainfall areas. Furthermore, breeding for an improvement in HI is also necessary for canola and mustard.
- Surprisingly, even under water stress conditions, canola and mustard crops failed to utilise the full amount of water present in the soil that could be related to inadequate root development or sensitivity to subsoil constraints. An improvement in subsoil water use may be an avenue for further improvements in yield and WUE of canola in this environment.

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