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Nitrogen efficiency of canola genotypes varies between vegetative stage and grain maturity

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Abstract There is no information on whether N efficiency in canola at maturity can be reliably determined by screening germplasm in the vegetative stage. Twelve canola genotypes identified in preliminary screening study as having either high or low N efficiency indices were tested for consistency in N efficiency between the vegetative stage and maturity. Plants were grown in a glasshouse under low or adequate N supply and N efficiency was assessed using the following criteria: dry weight at deficient N supply, relative yield at low vs. adequate N supply, and N utilisation efficiency. None of the 12 tested genotypes was classified as efficient or inefficient under all three criteria. One genotype (46C74) was classified as efficient under two criteria, and one genotype (Surpass 300 TT) was inefficient under two criteria. At maturity, three additional efficiency criteria were used: harvest index, N harvest index, and oil and protein concentration in seed. Two genotypes (Wesway and 46C74) (ranked as efficient at vegetative stage) remained efficient at maturity under most of the efficiency criteria used. On the other hand, genotype Surpass 603 CL ranked inefficient during the vegetative stage was ranked as efficient at maturity under two criteria. Overall, there was little consistency in the N efficiency ranking between vegetative stage and maturity in 12 tested genotypes. Screening canola germplasm for N efficiency for breeding purposes would therefore require an assessment at maturity.

Keywords Canola · Genotypic differences · Nitrogen deficiency · Nitrogen efficiency · Grain quality · Oil

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Introduction

Canola (*Brassica napus*), as the predominant oilseed crop in Australia, is grown on 1.4 million hectares annually. It is also the main oilseed crop in Western Australia, with 350,000 hectares grown annually (Carmody and Walton 2003). Canola production in Australia is constrained by poor soil fertility. In Western Australia, soils are among the most ancient and therefore heavily leached in the world, and are particularly deficient in P and N (Moore, 1998).

Selection and breeding of various crops has resulted in genotypes that respond to high fertilizer applications. In contrast, traits necessary for growth under limited nutrient supply have been suppressed or eliminated (Lynch 1998). Canola has a high demand for N (Holmes 1980); therefore, high rates of N fertilizer are usually applied to canola in order to obtain maximum seed yields, even though the contribution of

fertilizer-derived N to total N content is higher in vegetative than in reproductive shoot parts (Schjoerring et al. 1995). Canola has high N uptake efficiency from soil, but relatively low N utilisation efficiency (Rossato et al. 2001). Nitrogen uptake efficiency presents a quantitative measure of the actual uptake of N by the plant in relation to the amount of the N added to the soil (Zapata 1990). Nitrogen utilisation efficiency is calculated as total plant dry weight divided by N content (Siddiqi et al. 1989). Nitrogen efficiency decreases with an increase in N supply (Maman et al. 1999). Thus, fine-tuned N nutrition together with identification of more efficient genotypes could result in better use of N fertilizers (Mason and Brennan 1998) and may lead to an increase in crop productivity (Lynch 1998).

Genotypic differences in nutrient efficiency in various crops have been documented by many authors. Differences in P efficiency were demonstrated in wheat (*Triticum aestivum*) (Osborne and Rengel 2002) and maize (*Zea mays*) (Fageria and Baligar 1997). Differences in S efficiency in mustard (*Brassica juncea*) (Ahmad et al. 2005) and N efficiency in canola (*Brassica napus*) (Yau and Thurling 1987; Kessel and Becker 1999; Svecnjak and Rengel 2006; Balint et al. 2008) have also been found. Most of these authors reported that genotypic variations in utilization efficiency were more distinguishable under limited than under adequate nutrient supply.

In a study of 70 German canola genotypes from four different types (breeding lines, hybrids, and two resyntheses groups), significant differences were recorded in N efficiency, with the N harvest index as the most important assessment criterion (Kessel and Becker 1999). However, genetic variation in N efficiency could be multi-factorial and may vary with developmental stage (Seiffert et al. 1999). Factors such as total dry weight produced per unit of N content at flowering, N uptake after flowering, total dry weight produced per unit of N content of dropped leaves and N content of the seed have been used to assess N efficiency. Differential N efficiency among canola genotypes is not well understood, but it is likely that more than one mechanism is responsible for a given level of efficiency in a particular genotype (cf. Rengel 1999, 2001).

Genotypic differences in N efficiency (based on dry weight and N accumulation in the above-ground plant parts) during vegetative stage and grain maturity of 40 Australian genotypes were recorded (Yau and Thurling 1987). More recently, the focus was on modern Australian germplasm, including 84 canola genotypes (Svecnjak and Rengel 2006; Balint et al. 2008); however, all these studies concentrated on N efficiency during the vegetative stage. While it is advantageous for breeders to assess genetic differences in N efficiency during vegetative growth (the earlier, the better), there is no information on whether such an approach is likely to be relevant to N efficiency of canola genotypes at maturity. Hence, the aim of the present study was to evaluate whether there is consistency in N efficiency from the vegetative stage to physiological maturity in 12 canola genotypes shown in a preliminary study to differ in N efficiency at vegetative stage.

Materials and methods

Six efficient and six inefficient canola genotypes were tested for N efficiency during vegetative stage and at grain maturity. These chosen genotypes had either a high or low N efficiency indices at vegetative stage in preliminary N screening study of 84 canola genotypes (Balint et al. 2008).

A brown sandy soil (Uc4.22; Northcote 1971) was collected from a bushland site 15 km south-east of Lancelin (31°56′S, 115°20′E). Soil chemical characteristics of pH (H_2O) 5.9, 2% clay and 8 g/kg organic carbon with low availability of essential plant nutrients (N, K, P, Mg, S, Zn and Cu) made this soil suitable for the present study.

The soil was air dried and sieved to 2 mm. Basal nutrient and treatment salt solutions were added to soil, followed by air drying, thorough mixing, and filling into plastic pots lined with plastic bags (1,000 g of dry soil/pot). Basal nutrients were added at the following rates (mg/kg soil): 65.8 N; 87.6 K; 20.9 P; 54.2 S; 92.7 Na; 40.9 Ca; 179.4 Cl; 7.10 Mg; 3.25 Mn; 2.85 Zn; 0.62 Cu; 0.12 B; 0.09 Co; and 0.08 Mo. Nitrogen was supplied as NH_4NO_3.

The experiment was set up in a completely randomised block design with two treatments [adequate N (control) and deficient N] in six replicates. The initial rate of N of 65.8 mg N/kg soil for the control and 16 mg N/kg soil for the deficient N treatment was applied prior to sowing. Two subsequent N applications brought the total N applied to

140 mg N/kg soil for the adequate N treatment and 51 mg N/kg soil for the deficient N treatment by the time of vegetative harvest. Total amount of N applied to plants grown to grain maturity was 400 mg N/kg soil for the adequate and 180 mg N/kg soil for the deficient N treatment. Most N was applied during the rosette and the flowering and/or early grain filling stages. Potassium, S, P, and micronutrients were reapplied at the initial amounts at the rosette and the flowering stages.

The experiment was conducted in an evaporatively-cooled glasshouse in Perth as described elsewhere (Balint et al. 2008) in early spring with average day/night temperatures of 25/14°C. Canola genotypes were sown at ten seeds per pot in March and thinned to two plants 8 days after sowing. Pots were watered with deionised water daily and weighed to field capacity (10% w/w) every second day. Insect pests were controlled using standard chemical treatments as required.

One canola shoot in each replicate was harvested by cutting the plant just above the soil surface 42 days after sowing when growth reduction and other N deficiency symptoms were evident in most genotypes in the deficient N treatment. Shoot dry weight was determined after drying in an oven at 65°C for 7 days. Nitrogen content was determined on a 4-channel Autoanalyzer system, using modifications of manufacturer's procedures (Anon 1979). After digestion with sulphuric acid and hydrogen peroxide, N was determined colourimetrically by the indophenol blue method (Yuen and Pollards 1954).

The remaining plant in each replicate was harvested at grain maturity (early maturing genotypes were Surpass 300 TT, Surpass 603 CL, and Bugle; late maturing were CBWA 005, Taparoo, 46C74, and Wesway). Dropped leaves were collected throughout the growth period. Dry weights of stem, siliques and dropped leaves were determined after drying in an oven at 65°C for 3 days. The N content in dropped leaves, stems, and siliques was measured as mentioned above. Oil and protein concentration in seed was determined in intact seeds using near infrared spectrometry (NIR Systems 6500).

The N efficiency in vegetative stage and grain maturity was evaluated using the following three criteria: (1) growth (assessed as dry weight) at deficient N supply; (2) dry weight at deficient relative to adequate N supply; (3) N utilisation efficiency, expressed as shoot (for vegetative stage) and/or seed (for maturity stage) dry weight per unit of N content in shoots and/or seeds. In addition, the harvest index (HI) and N harvest index (NHI) parameters were used for N efficiency evaluation at the grain maturity stage. Harvest index was defined as the ratio of grain biomass to the total above-ground biomass at harvest. NHI was defined as the ratio of N in grain to N in total above-ground biomass at harvest.

The N efficiency ranking (I, inefficient; M, medium efficient; E, efficient) was calculated according to Rengel and Graham (1995); boundaries for the medium efficiency interval were formulated by subtracting or adding the value of one standard error from the median point of a particular efficiency criterion. The data were analysed by ANOVA using GENSTAT 6.1 statistical program.

Results and discussion

Genotypic differences in N efficiency in canola have been documented for two Canadian (Grami and La Croix 1977) and 40 Australian genotypes (Yau and Thurling 1987). In the study of 70 German canola genotypes, N harvest index was chosen as the most important assessment criterion (Kessel and Becker 1999). More recent work on differential N efficiency in canola assessed four commercial cultivars (Svecnjak and Rengel 2006) and 84 genotypes (Balint et al. 2008). The reasons for the genotypic differences were related to uptake, transport and/or utilisation of nutrients within the plant, but the underlying mechanisms were not elucidated.

Plant growth is one of the most commonly assessed indicators of severity of stress related to nutrient deficiency (Rengel 1999, 2001). In the present study, the first indicators of N deficiency problems in canola shoots were visually detectable 20 days after sowing, manifested by reduced growth and change in the leaf colour from dark to pale green (chlorosis) only in plants grown under deficient N supply (51 mg N/kg of soil during the vegetative stage). As the plants grew older, the deficiency symptoms became more severe, with purple colouration developing on the upper surface of the leaves. Similar deficiency symptoms in canola plants were reported by Pluske and Osborne (2001). The extent of purple pigmentation under N deficiency varied among genotypes.

Vegetative stage

Effect of N supply on dry matter production

Canola genotypes differed considerably in growth and N uptake parameters. Shoot dry weight was influenced by N treatment, but also by differences among genotypes, whereas the N treatment × genotype interaction was not significant (Table 1). Dry matter production under adequate N supply was approximately 3.5 times higher than under deficient N supply (data not shown). In comparison, the amount of N applied in the adequate N treatment was 2.8 times higher than in the deficient N treatment, indicating higher N utilisation efficiency at deficient compared to adequate supply (Table 1). Similar findings were reported by Yau and Thurling (1987) and Svecnjak and Rengel (2006).

Dry matter production under deficient N supply ranged between 550 and 900 mg shoot^{-1} (Fig. 1). Three canola genotypes (Wesway, BLN 331, and Chikuzen) had dry matter production above 750 mg shoot^{-1} in the deficient N treatment and were identified as efficient. These genotypes had approximately 2-fold higher dry matter production than genotypes CBWA 005, Eyre, and Surpass 300 TT that were considered inefficient. A similar range of growth differences in canola genotypes under limited N supply were reported by Svecnjak and Rengel (2006).

Concentration of nitrogen in canola shoots

Nitrogen concentrations in shoots were significantly affected by the N treatment × genotype interaction (Table 1). Nitrogen concentration in shoots of canola genotypes grown under deficient N supply ranged from 16.5 (genotype 46C74) to 23.6 g N/kg shoot DW (genotype CBWA 005) compared with plants grown under adequate N supply, with N concentration in shoots ranging from 20.8 (genotype Chikuzen) to 32.2 g N/kg shoot DW (genotype Bugle). Canola genotypes under adequate N supply (140 mg N/kg soil supplied during the vegetative stage) accumulated

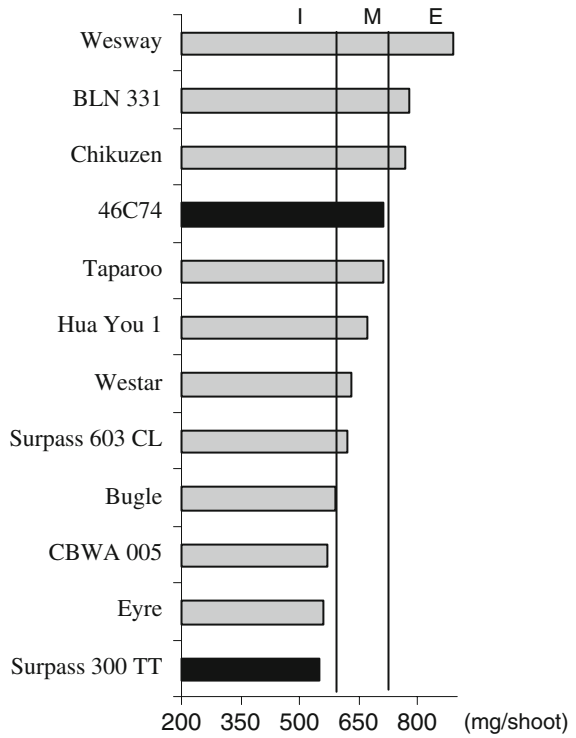

Fig. 1 Shoot dry weight of 12 canola genotypes grown at deficient nitrogen supply (51 mg N/kg soil) for 42 days. Genotypes are ranked in the order of decreasing nitrogen efficiency (E, efficient; M, medium; I, inefficient). The boundaries for the medium efficiency interval were created by subtracting or adding the value of one standard error to the median point of the efficiency criterion. Black bars represent the most (46C74) and least efficient genotypes (Surpass 300 TT) based on the combination of three N efficiency criteria in the vegetative stage

Table 1 Analyses of variance for dry weight, N concentration and content in shoot, and N utilisation efficiency of canola genotypes in the vegetative stage

	Genotype (G)	Nitrogen treatment (N)	G X N
Shoot dry weight (mg shoot^{-1})	**	***	NS
N concentration in shoots (g N/kg shoot DW)	***	***	**
N content in shoots (mg N shoot^{-1})	***	***	*
N-utilisation efficiency (g shoot DW/g plant N)	***	***	*

NS: not significant; *, **, *** Significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$, respectively

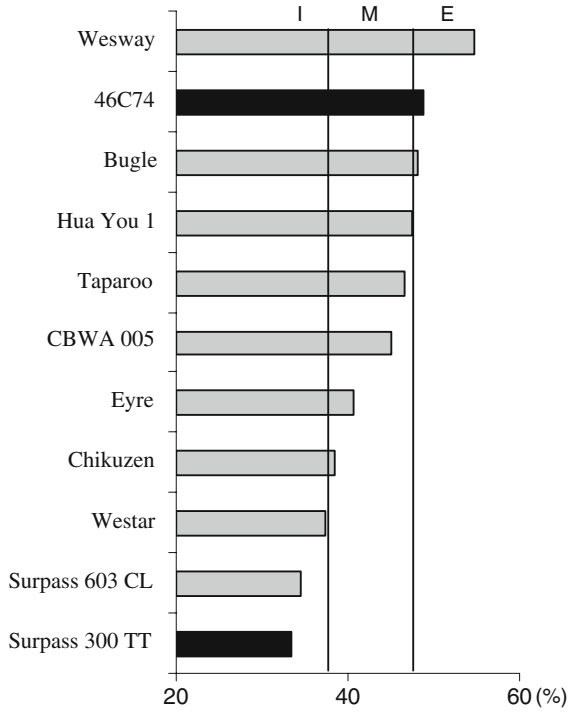

Fig. 2 Ranking of 12 canola genotypes based on the ratio between shoot dry weight at deficient (51 mg N/kg soil) and adequate N supply (140 mg N/kg soil) after 42 days of growth. For efficiency intervals (E, efficient; M, medium; I, inefficient) and genotypes singled out with black bars see Fig. 1

19–32 mg N/shoot, and only 20% less (16–22 mg N/shoot) under deficient N supply (51 mg N/kg soil during the vegetative stage). It is worth noting again that the amount of N applied in the adequate N treatment was 2.8 times higher than that applied in the deficient N treatment. Canola genotypes under adequate N supply accumulated in shoots approximately 23% of supplied N compared with the deficient N treatment where plants utilised 50% of the supplied amount. Genotypes Bugle and CBWA 005 (ranked as inefficient under all other efficiency criteria) accumulated significantly higher amounts of N per shoot than genotypes 46C74, Wesway and Chikuzen (ranked as efficient under most of the efficiency criteria) in the deficient N treatment (data not shown).

Dry matter production at deficient relative to adequate N supply

Dry matter production at deficient vs. adequate N supply (relative yield) was approximately 35%. Genotypes with the relative yield higher than 47% were rated efficient (Fig. 2). The efficient genotype 46C74 had small differences in dry matter production between adequate and deficient N supply compared with inefficient genotype Surpass 300 TT for which 3-fold differences in growth between the two N treatments were recorded.

Nitrogen utilisation efficiency

This index reflects how efficient canola genotypes were in converting N taken up into organic matter. Significant differences were recorded in genotype × N treatment interaction (Table 1), suggesting that genotypes that utilised N more efficiently under limited supply were not necessarily the more efficient ones under adequate supply. For example, genotype Wesway was superior to genotype Westar under N deficiency but not under N sufficient supply (Fig. 3), making these two genotypes valuable for a further study on mechanisms of N efficiency.

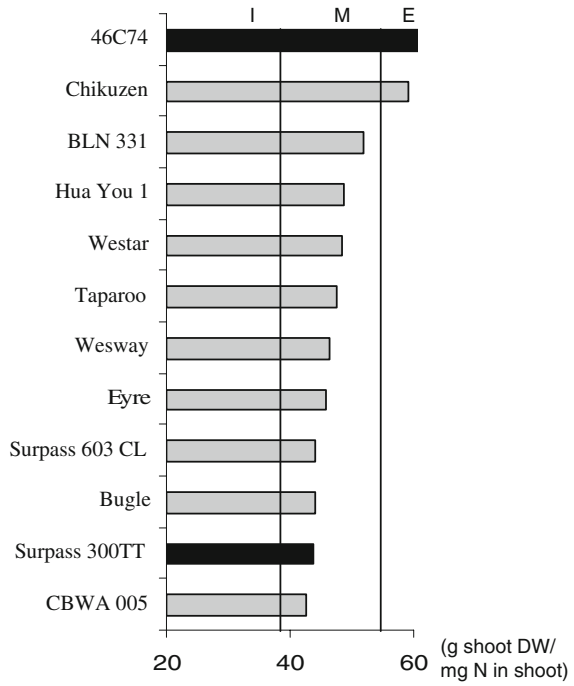

Fig. 3 Nitrogen utilisation efficiency [calculated as the amount of shoot dry weight (DW) produced per unit of nitrogen in shoots] of 42-day-old canola genotypes grown at deficient nitrogen supply (51 mg N/kg soil). For efficiency intervals (E, efficient; M, medium; I, inefficient) and genotypes singled out with black bars see Fig. 1

The N utilisation efficiency ranged between 42 and 62 g shoot DW/g plant N (Fig. 3). Two canola genotypes (46C74 and Chikuzen) were rated as efficient, with N utilisation efficiency index above 60 g shoot DW/g plant N (Fig. 3).

Considerable genetic variation in N efficiency was identified in canola genotypes by Yau and Thurling (1987) and Svecnjak and Rengel (2006). Increased efficiency of nutrient use in plants may be due to an increasing rate of nutrient transport within the plant or specific compartmentation in cells (Rengel and Graham 1995; Rengel and Hawkesford 1997); variations in these mechanisms may contribute to genotypic differences in canola genotypes recorded in the present study.

Nitrogen efficiency based on all three criteria combined

None of the 12 tested genotypes was classified as efficient or inefficient under all three criteria. One genotype was classified as efficient under two criteria (46C74) and one genotype as inefficient under two criteria (Surpass 300 TT).

Conclusion

Canola genotypes significantly differed in N efficiency during vegetative stage. Differential N efficiency of canola genotypes from the preliminary screening study was confirmed, with six efficient and six inefficient genotypes registering such status based on at least one efficiency criterion. If the efficiency ranking among 12 canola genotypes would remain the same at grain maturity stage, screening a large number of canola genotypes during vegetative stage would be sufficient for selection purposes in developing improved genotypes for low-input canola production.

Grain maturity

Effect of N supply on growth

Symptoms of N deficiency were apparent during the period of rapid growth, during stem elongation, and before and after flowering. Affected plants had pale green leaves. Flowering in the deficient N treatment started approximately 10 days later than in the adequate N treatment. Flowers in the deficient N treatment were pale creamy yellow, compared with bright yellow flowers in the adequate N treatment. In the deficient N treatment there was reduced silique set as well as some silique abortion. Also, leaves, stems and in some cases siliques had purple colouration and the leaves were thick and small in size. The extent of purple pigmentation varied among genotypes.

Canola genotypes differed considerably in growth and N uptake at grain maturity. Dry matter of stems, leaves, siliques, and seeds was strongly influenced by genotypic differences. The N treatment effect was significant for dry weight of stems and seeds only. Significant genotype × N treatment interaction for dry weight was recorded only for the stems (Tables 2, 3). Dry matter production of whole plants under adequate N supply (400 mg N/kg soil for the whole growth cycle) was on average 20% higher than that under deficient N supply (data not shown). In contrast, the amount of N applied in the deficient N treatment was more than 2-fold lower (180 mg N/kg soil) in comparison with adequate N supply.

Seed yield at deficient N supply was approximately 35% of that at adequate N supply. Similar variations in seed production under deficient and sufficient N supplies were reported by Svecnjak and Rengel (2006) for four canola genotypes. Seed production under limited N supply ranged between 50 and 250 mg plant^{-1} (Fig. 4a). Four canola genotypes (Surpass 300 TT, Surpass 603 CL, Bugle, and Wesway) produced more than 180 mg seeds plant^{-1} and were ranked efficient. These genotypes had approximately 2-fold higher seed production than inefficient genotypes Chikuzen, Taparoo, and Hua You 1 that produced less than 74 mg seed plant^{-1}.

Dry matter production of stems under deficient N supply ranged from 300 to 900 mg plant^{-1}. It was significantly influenced by genotype ($P < 0.001$); Surpass 603 CL or CBWA 005 had considerably higher dry matter production than Surpass 300 TT or 46C74. Nitrogen treatments also had a significant effect on dry matter production of stems ($P < 0.01$). In the present study, the significant interaction genotype × N treatment for dry matter production was only recorded for stems.

Dry weight of dropped leaves under deficient N supply ranged from 10 to 40 mg plant^{-1}. No main effects ($P = 0.06$–0.36) nor interaction ($P = 0.14$) was significant.

Table 2 Analyses of variance for dry weight, N concentration and N uptake of stems, siliques and dropped leaves of canola genotypes at maturity

	Genotype (G)	Nitrogen treatment (N)	G × N
Stem			
Stem dry weight (mg plant^{-1})	***	**	**
N concentration in stems (g N/kg stems DW)	***	***	***
N content in stems (mg N stems^{-1})	***	***	***
Siliques			
Siliques dry weight (mg plant^{-1})	*	NS	NS
N concentration in siliques (g N/kg siliques DW)	***	***	***
N content in siliques (mg N siliques^{-1})	***	***	***
Dropped leaves			
Dry weight of dropped leaves (mg plant^{-1})	NS	NS	NS
N concentration in dropped leaves (g N/kg DW)	NS	**	NS
N content in dropped leaves (mg N leaves^{-1})	NS	***	NS

NS: not significant; *, **, *** Significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$, respectively

Table 3 Analyses of variance for seed dry weight, N concentration and content in seed and N utilisation efficiency of canola genotypes at maturity

	Genotype (G)	Nitrogen treatment (N)	G × N
Seed dry weight (mg plant^{-1})	***	*	NS
N concentration in seeds (g N/kg seeds DW)	***	***	NS
N content in seeds (mg N seed^{-1})	***	***	NS
N-utilisation efficiency in seeds (g DW of seeds/N in seeds)	***	***	*
Oil concentration in seed (g kg^{-1})	***	***	NS
Protein concentration in seed (g kg^{-1})	***	***	NS

NS: not significant; *, *** Significant at $P = 0.05$ and $P = 0.001$, respectively

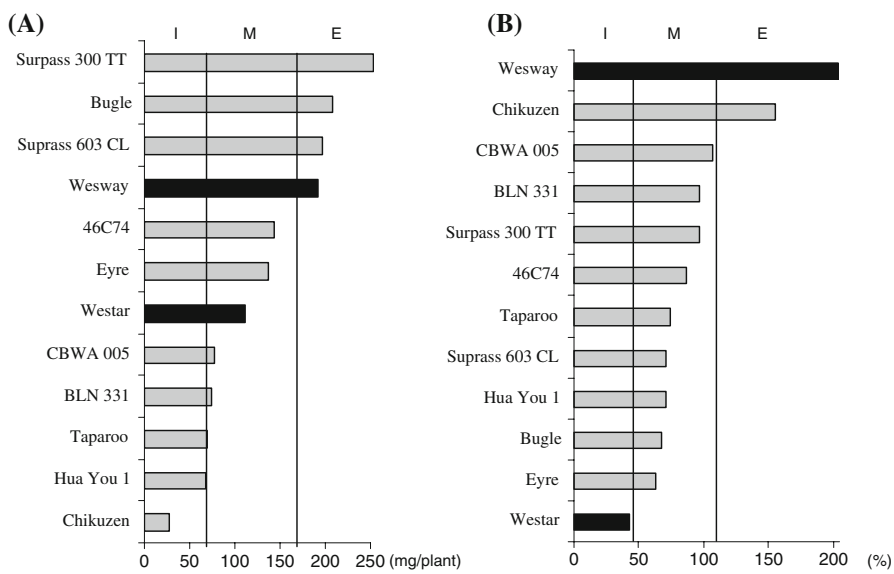

Fig. 4 Seed dry weight (**a**) and ranking of 12 canola genotypes based on the ratio (**b**) between total seed dry weigh at deficient (180 mg N/kg soil) and adequate nitrogen supply (400 mg N/kg soil) at grain maturity. The boundaries for the medium efficiency interval were formulated by subtracting or adding the value of one standard error to the median point of the efficiency criterion. Black bars represent the most (Wesway) and least efficient genotypes (Westar) based on the combination of N efficiency criteria at maturity

Dry matter production of siliques ranged from 98 mg plant^{-1} (Chikuzen) to 220 mg plant^{-1} (Surpass 300 TT) under deficient N supply. A significant effect on dry matter production in siliques was recorded for the genotype treatment only.

Nitrogen concentration in dropped leaves, stems, siliques, and seeds

Nitrogen concentration in siliques ranged from 4.5 to 10 g N/kg DW (BLN 331 < CBWA 005) at deficient N supply; and from 12.4 to 23.8 g N/kg DW (46C74 < Surpass 300 TT) at adequate N supply. Concentration of N in stems was significantly affected by the N treatment. It was also affected by the N treatment × genotype interaction, indicating differential genotypic response at the two levels of N nutrition (Table 2). Concentration of N in stems ranged from 13.7 to 26.7 under adequate and from 3.9 to 7.9 g N/kg DW under deficient N supply. These concentrations of N under deficient N supply were lower than the concentrations in shoots during the rosette stage (16–23 g N/kg). The majority of N content from shoots was remobilized to seeds as the largest sink (around 70% of shoot N, data not shown). These findings are consistent with Rossato et al. (2001) who suggested that most of the N used for grain filling was derived from mobilization of N stored in vegetative tissues (ie. shoots).

Canola genotypes grown under adequate N supply (400 mg N/kg soil during the whole growth cycle) accumulated 19–32 mg N/stem, and only 20% less (16–22 mg/stem) under deficient N supply (180 mg N applied/kg soil) (data not shown, see Table 2 for statistics). In contrast, N application in the adequate N treatment was 2.1 times higher than in the deficient N treatment, suggesting that plants under adequate N supply were less efficient users of N than plants supplied deficient amounts of N.

Genotypes Bugle and CBWA 005 accumulated significantly higher amounts of N in stems than genotypes 46C74, Wesway and Chikuzen under deficient N supply. Hence, genotypes Bugle and CBWA 005 may not be efficient in remobilizing N towards the sink organs (seed). The two groups of genotypes differing in the N content in stems might differ in the type and/or amounts of amino acids involved in transporting N-containing compounds. The predominant amino acid in canola phloem is glutamine (Mollers et al. 1996). However, genotypes more efficient in remobilizing N might contain greater amounts or proportions of amino acid asparagine, the more efficient N transporter with the N:C ratio of 0.5 compared to 0.4 for glutamine (Seiffert et al. 1999).

Nitrogen concentration in dropped leaves of plants grown under deficient supply ranged from 5.2 to 5.9 g N/kg DW; these values are similar to the ones obtained by Svecnjak and Rengel (2006) and Hocking et al. (1997). Genotype Surpass 300 TT (ranked as inefficient at vegetative stage) had the highest N concentration in dropped leaves (making this genotype relatively inefficient due to N losses via leaf drop). In contrast, N concentration in leaves at adequate N supply ranged from 9.8 to 14.2 g N/kg DW; these values are higher than mentioned in the earlier reports (Svecnjak and Rengel 2006; Hocking et al. 1997). This discrepancy might be due to higher amounts of N applied in our study compared with the earlier reports, suggesting that canola could take up N in high amounts when supplied luxuriously, but the remobilization of N from leaves remained low.

Under deficient N supply, canola seeds had N concentrations from 37.4 (Wesway) to 42.7 g N/kg DW (Westar), whereas seeds at sufficient supply had N concentration from 45 (CBWA 005) to 50.5 g N/kg DW (Surpass 300 TT). These differences in seed N concentrations were proportionally smaller than differences in N applications, making genotypes under deficient N supply more efficient in using available N compared with genotypes in the adequate N treatment. Nitrogen content in seed under deficient N supply ranged from 33 (genotypes BLN 331 and Hua You 1) to 100 mg N/seeds (Surpass 300 TT).

Nitrogen concentration in siliques under the adequate N supply ranged from 45 (BLN 331) to 93 g N/kg DW (genotype CBWA 005). Nitrogen content ranged from 5.3 (BLN 331) to 16.5 mg N/silique (Surpass 300 TT) (data not shown).

Nitrogen accumulation per whole above-ground plant under deficient N supply ranged from 105 (Chikuzen) to 165 mg N/plant (Surpass 603 CL) (Fig. 5). There was no similarity among genotypes in amounts of N accumulated per plant parts. Genotypes Hua you 1 and CBWA 005 lost more than Surpass 603 CL and Chikuzen by dropping off N-rich leaves, suggesting poor N remobilization rate from leaves of Hua you 1 and CBWA 005 (Fig. 5). Genotype Surpass 603 CL took up more N from the soil than

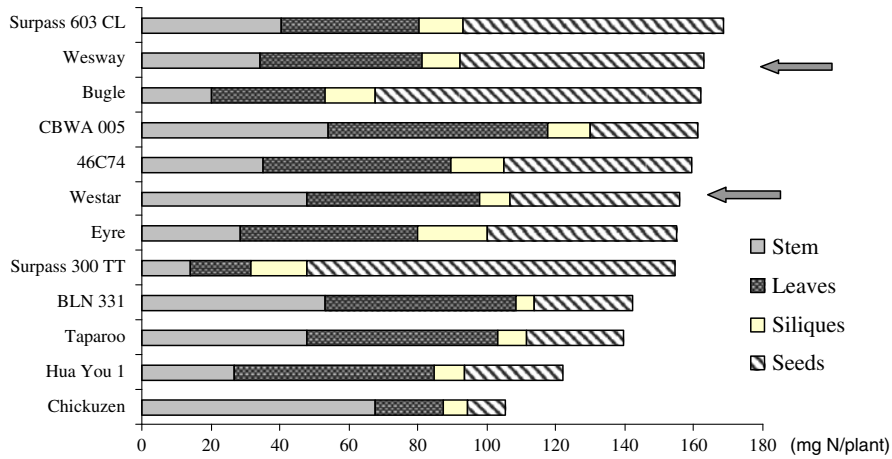

Fig. 5 Nitrogen content of stems, leaves, siliques and seed of 12 canola genotypes grown at deficient nitrogen supply (180 mg N/kg soil) at grain maturity

any other genotype under deficient N supply; interestingly, this genotype was ranked as inefficient at the vegetative stage. Even though the largest N accumulation in canola shoots was reported to occur during the flowering stage (Chamoro et al. 2002) or before anthesis (Hocking et al. 1997), the largest N accumulation in Surpass 603 CL occurred during flowering (data not shown).

Growth at deficient relative to sufficient nitrogen supply

Relative seed yield at low N supply was 47–200% of that at sufficient N supply. Genotypes with relative seed yield higher than 112% were rated efficient, whereas genotypes with relative seed yield lower than 49% were recognized as inefficient (Fig. 4b). Genotype Wesway (ranked as efficient under this assessment criterion) had smaller differences in seed production between adequate and low N supply compared with inefficient genotype Westar for which 3-fold differences in growth were observed between the two N treatments. Genotypes Wesway and CBWA 005 yielded more seed under deficient than under adequate N supply, even though seed yield of these two genotypes was lower than in other tested genotypes.

Nitrogen utilisation efficiency

Nitrogen efficiency in stem dry matter production was influenced by the main effects of genotype ($P < 0.005$) and N treatment ($P < 0.001$). There was the significant genotype × N treatment interaction ($P < 0.005$) (Table 2). Nitrogen utilisation efficiency in stems under deficient N supply ranged from 116 to 210 g DW/mg N. Genotypes with an efficiency index above 189 g DW/mg N were ranked as efficient (ie. genotype Wesway). Three canola genotypes (Westar, Taparoo, and Chikuzen) had efficiency indices below 145 g DW/mg N and were ranked as inefficient (data not shown).

Nitrogen efficiency in seed (seed dry weight per unit of N accumulated in the seed) was influenced by the main effects of genotype ($P < 0.001$), N treatment ($P < 0.001$) and the genotype × N treatment interaction ($P < 0.013$) (Table 3). Nitrogen efficiency index in seeds under sufficient supply ranged from 19.8 to 22 g DW/mg N. There was only a 17% difference between the two N treatments (indices under deficient supply ranged from 23.5 to 26.8 g DW/mg N). A similar decrease in N efficiency by increasing N nutrition rates were reported elsewhere (Hocking et al.1997). Two canola genotypes (BLN 331 and Wesway) were rated as efficient, with the N efficiency index above 25.7 g DW/mg N (Fig. 6). Other canola genotypes were ranked as medium efficient (Fig. 6).

Harvest index and nitrogen harvest index

Harvest index under deficient N supply ranged from 2.9 to 18.5%. Genotypes with a harvest index above 15.4% (Surpass 300 TT and Wesway) were ranked as efficient, whereas genotypes with a harvest index below 8.1% (Chikuzen and Taparoo) were ranked as inefficient (Fig. 7a). Two different N rates in this study had no effect on the harvest index (data not shown). Harvest index values in our

study were lower than those obtained by Svecnjak and Rengel 2006 (average 22%) or Hocking et al. 1997 (27–34%) due to poor performance (low seed yield) of some genotypes (Chikuzen, Hua You 1 and Taparoo).

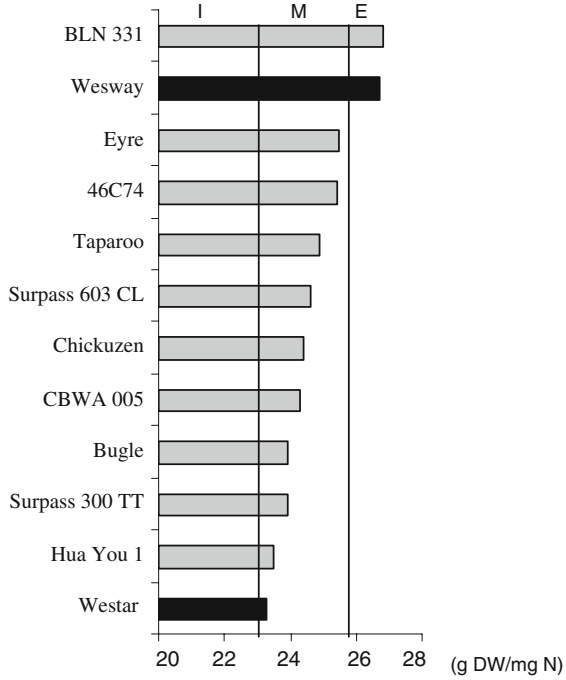

Fig. 6 Nitrogen utilisation efficiency of seeds [calculated as the amount of seed dry weight (DW) produced per unit of nitrogen in seeds] of canola plants grown at deficient nitrogen supply (180 mg/kg soil). For efficiency intervals (E, efficient; M, medium; I, inefficient) and genotypes singled out with black bars see Fig. 4

Nitrogen harvest index ranged from 17.5% (Chikuzen and Taparoo) to 73.4% (Surpass 300 TT and Bugle) for genotypes grown under deficient N supply (Fig. 7b). Nitrogen harvest index in canola is usually low because of dropping off N-rich leaves (Rossato et al. 2001). In our study, genotypes Surpass 300 TT and Bugle had relatively high seed yield and low dry matter production of leaves under deficient N supply that contributed to higher N harvest index (compared to genotypes Chikuzen and Taparoo that had poorer seed yield with a higher proportion of dry matter in dropped leaves).

Oil and protein concentration in seed

Oil concentration in seed was influenced by the main effects of genotype ($P < 0.01$) and N treatment ($P < 0.01$), but the genotype × N treatment interaction was non-significant ($P = 0.35$) (Table 3). In seed under deficient N supply oil concentration ranged from 435 to 475 g kg^{-1} for different genotypes. Genotypes with oil concentration above 465 g kg^{-1} (Surpass 300 TT, Surpass 603 CL, BLN 331 and Wesway) were ranked as efficient, whereas genotypes with oil concentration below 448 g kg^{-1} (Westar and Hua You 1) were considered inefficient (Fig. 8a). On the other hand, oil concentration in seed

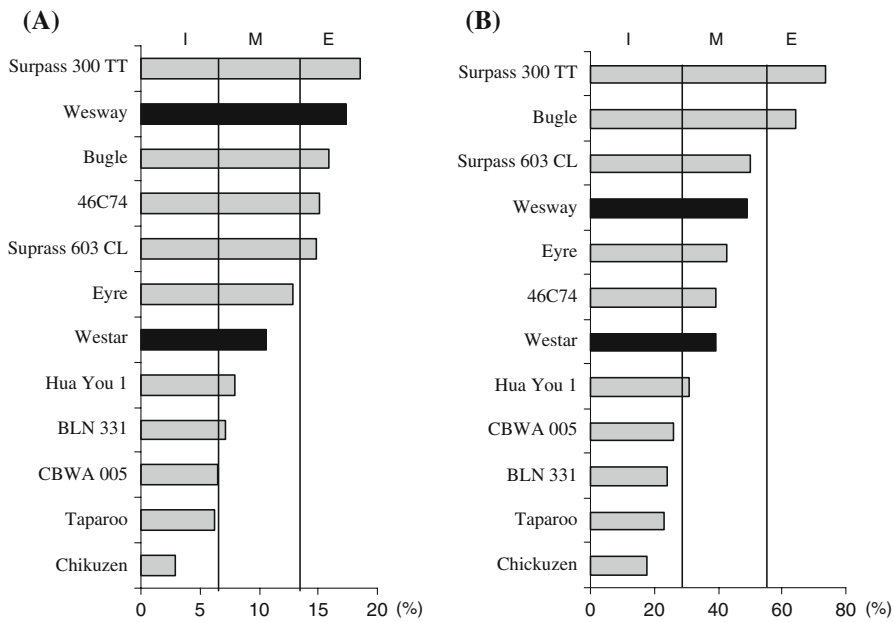

Fig. 7 Harvest index (**a**) [(seed DW/above-ground DW) × 100%] and nitrogen harvest index (**b**) [(seed N content/above-ground N content) × 100%] of 12 canola genotypes grown at deficient nitrogen supply. For efficiency intervals (E, efficient; M, medium; I, inefficient) and genotypes singled out with black bars see Fig. 4

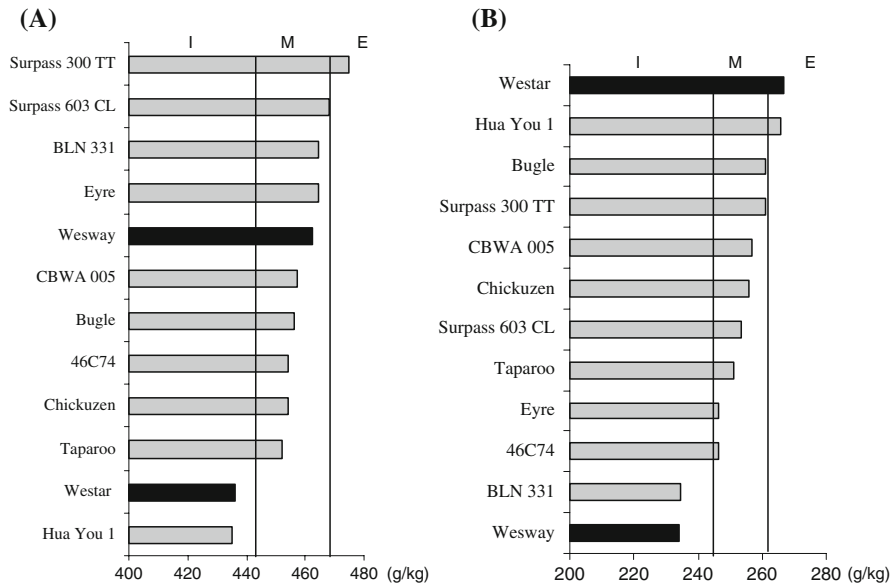

Fig. 8 Oil (**a**) and protein (**b**) concentration in seeds of 12 canola genotypes grown at deficient nitrogen supply (180 mg N/kg soil). For efficiency intervals (E, efficient; M, medium; I, inefficient) and genotypes singled out with black bars see Fig. 4

under adequate N supply ranged from 414 to 440 g kg^{-1}.

Protein concentration in seed was influenced by the main effects of genotype ($P < 0.01$) and N treatment ($P < 0.01$), but the genotype × N treatment interaction was non-significant ($P = 0.18$) (Table 3). Protein concentration in seed for the deficient N treatment ranged from 234 to 264 g kg^{-1}. Genotypes with protein concentration above 262 g kg^{-1} (Westar and Hua You 1) were ranked efficient, whereas genotypes with protein concentration <247 g kg^{-1}(Wesway, Eyre, 46C74 and BLN 331) were ranked as inefficient (Fig. 8b). Protein concentration for the adequate N treatment ranged from 282 to 315 g kg^{-1}.

The association between oil and protein concentrations in seed is generally negative, ie. if concentration of oil in seed increases, protein concentration decreases and vice versa (Grami et al. 1997, Pritchard et al. 2000). In our study, two different N applications produced the same negative correlation of protein and oil concentration in seed as explained above (e.g. high N application increased protein and decreased oil in seed of Wesway). Under deficient N supply, this genotype produced seed with low protein, but high oil concentration (data not shown). Similar findings were reported for different N applications in the field (Brennan et al. 2000). The sum of oil and protein concentration in seed was 620 g kg^{-1} of field-grown canola in Victoria (Pritchard et al. 2000) and Western Australia (Brennan et al. 2000). In our glasshouse study this sum was approximately 690 g kg^{-1}; this value might be higher than that obtained by Pritchard et al. (2000) and Brennan et al. (2000) due to an impact of favourable glasshouse conditions during the seed filling stage in contrast to field conditions.

Conclusions

Canola genotypes differed significantly in N efficiency at grain maturity. Two genotypes (Wesway and 46C74) (ranked as efficient at vegetative stage) remained efficient at maturity under most of the efficiency criteria used. On the other hand, genotype Surpass 603 CL (inefficient during vegetative stage) was ranked as efficient genotype at maturity under two criteria.

Overall, there was little consistency in the N efficiency ranking from vegetative stage to physiological maturity in 12 tested genotypes. Thus, vegetative-stage assessments have poor predictive ability with respect to N efficiency at maturity and cannot be used as an efficient selection tool for N efficiency of canola cultivars.

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