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*Full Length Research Paper*

# Carbon and nitrogen stoichiometry in *Brassica napus* L. seedlings after supplementation with $Ca^{2+}$ and $K^+$ under irrigated and drought stress conditions

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**Exposure of plants to long periods of water scarcity, mainly in arid and semi-arid regions, is one of the major reasons for over 50% reduction in average yields. Proper management of *Brassica napus to* enhance its ability to survive under drought and high temperature stress at early growth stages, besides development of tolerant genotypes, could improve its production in the rainfed areas. In the present study, we report the interactive effects of $Ca^{2+}$, $K^+$ and N supplementation and water availability on the fresh biomass, N and C content, as well as C/N ratio of crop plants. Exposure to water stress significantly reduced the fresh biomass, Nitrogen (N), Carbon (C) content and the C/N ratio. $Ca^{2+}$ and $K^+$ supplementation before drought positively affected fresh biomass by stimulating N uptake and C assimilation. However, the C/N ratio was reduced after supplementation with $Ca^{2+}$ and $K^+$. The N supplementation before drought imposition, though enhanced the N uptake, but the excessive damage to cell membranes and electrons leakage from Electron Transport Chain (ETC) during photosynthesis resulted in a decrease in C assimilation. Consequently, there was a decrease in the C/N ratio in seedlings exposed to drought after supplementation with N.**

**Key words:** Drought, nutrients supplementation, carbon, nitrogen, C/N ratio.

## INTRODUCTION

Water is known to be one of the main limiting factors affecting the plants yield, growth and development. The plants are naturally exposed to long periods of water scarcity stress, mainly in arid and semi-arid regions, which is one of the major reasons for over 50% reduction in average yields of crops (Wang et al., 2003). Periods of drought in the winter and early spring affect the development of *Brassica napus* L. at the shooting stage. However, the temperature and water stress occurring at the earlier growth stages in this crop, unlike in the flowering stage, seemed to exhibit recovery in the seed yield (Müller et al., 2010). The proper management of *Brassica napus* to enhance its ability to survive under drought and high temperature stress at early growth stages could improve its production in the rainfed areas. Several physiological and biochemical changes occur in plants as adaptive responses to various abiotic stresses including an increase in leaf osmotic potential, activation of high affinity nutrient transporters, remobilization of nutrients from the older to younger leaves, retardation of growth and photosynthesis and changes in pigment composition (Diaz et al., 2006; Sagi et al., 1997; Sharkey and Schrader, 2006). Similarly, drought stress can create imbalance in the nutrients composition of plants by disturbing the uptake of different nutrients (Dambrine et

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al., 1993). Furthermore, reduction in transpiration also restricts the nutrients translocation to the shoots due to restricted active transport and membrane permeability (Alam, 1999; Viets, 1972). This reduced translocation of nutrients acts as signals within the plants for adaptation to drought, thus the reduced availability of different mineral elements and drought act synergistically in root to shoot signaling.

Nitrogen (N), a key element for plants, is obtained from the rhizosphere through the roots. It is often a limiting factor for plant growth and development and is closely correlated with Carbon (C) assimilation. Large leaf N content was linked to a high stomatal ($g_s$) and mesophyll conductance ($g_m$). Analysis using the $C_3$ photosynthesis model indicated that $CO_2$ assimilation tended to be limited by RuBP carboxylation in plants grown at low N concentration, whereas it was limited by RuBP regeneration in plants grown at high N concentration. N nutrition generally alters N allocation between photosynthetic components. Contents of leaf N, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and cytochrome *f* (cyt *f*) increased with increasing N supply, but the cyt *f*/Rubisco ratio decreased (Hirel and Lea, 2002; Hodges, 2002; Stitt et al., 2002). The C/N interaction takes place within a context of energy use and production involving cooperation between different sub-cellular compartments (Foyer et al., 2003; Guo et al., 2005).

Under dry conditions, N use efficiency is low, especially influenced by seasonal drought and unbalanced fertilization (Wang et al., 2007a,b). Rockström and Barron (2007) suggested that yield response to rain was only achieved through proper soil fertility management. Similarly, $Ca^{2+}$ and $K^+$ are important plant nutrients involved in cell signaling, membrane stability, osmotic adjustment and protein stabilization. In droughted *Vicia faba*, it was found that percentage leakage and the efflux of $K^+$ and $Ca^{2+}$ were remarkably higher than those of the control plants; high leakage may lead to depletion of $K^+$ and other constituents leading to subsequent disturbances in plant growth and metabolism (Basset, 1998). Also, $Ca^{2+}$ was found to protect *Chlorella* cells as well as reducing $K^+$ efflux in case of cell dehydration (Basset and Issa, 1994). Furthermore, while N or water deficits usually decrease the rate of C assimilation (*A*), N limitation per se may affect stomatal behaviour in different ways; both increases (Livingston et al., 1999) and decreases (Lima et al., 1999) in stomatal conductance (gs) have been noted. Thus, appropriate fertilization can mitigate the adverse effect of drought on crop plants. However, very little information is available on the interactive effect of $Ca^{2+}$, $K^+$ and N supplementation and water availability on the N and C content as well as C/N ratio of crop plants. In the present experiment, the interactive effects of supplemental $Ca^{2+}$, $K^+$ and N on the tissue C and N stoichiometry under irrigated and drought stress conditions were investigated.

## MATERIALS AND METHODS

### Plant material and experimental layout

The experiment was performed at the Institute of Biotechnology and Genetic Engineering, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during March-July, 2010. *B. napus* L. seedlings were grown in plastic pots filled with 6 Kg of soil and farm yard manure (1:1). The average $K^+$, N and $Ca^{2+}$ content of this soil mixture was 0.013, 0.61 and 0.006%, respectively with a field capacity of 33 %. The seedlings were grown under natural light in a greenhouse. Thinning was done one week after germination and five seedlings of uniform size were maintained in each pot. One month old plants were left as such or supplemented with either 30, 60, 90 mM of $Ca(NO_3)_2.4H_2O$ (C 30, C 60 and C 90) or 50, 100, 150 mM $KNO_3$ (K 50, K 100 and K 150) or 30, 50, 100mM $NH_4NO_3$ (N 30, N 50 and N100). After one week of supplementation, the plants were divided into two groups; half were kept at field capacity by irrigating daily. Drought stress was imposed by withholding water from half the pots for 10 days. Five pots were maintained in each treatment and analyses were performed in triplicate after 10 days of drought imposition.

### Elemental analysis

N and C content were determined by “CHNS elemental Analyzer” (Vario EL III, Germany). Briefly, the samples were dried at 110°C, crushed, ground, packed in dry tin container and were dropped into a vertical quartz tubes maintained at 1150°C through which constant flow of helium gas occurred at 200 ml/min. When the samples were introduced, the helium stream was temporarily enriched with pure oxygen for flush combustion to occur. The mixture of gases so obtained was passed over $Cr_2O_3$ to obtain quantitative combustion and over copper at 650°C to remove excess oxygen and reduce oxide of nitrogen to $N_2$. Finally, the gas mixture was passed through a chromatographic column heated to about 100°C. The individual components ($N_2$ and $CO_2$) were separated and eluted to a thermal conductivity detector (TCD). The detector signal was fed to potentiometric and recorded in parallel with an integrator and digital printout (AOAC, 2000).

### Statistical analysis

Statistical analyses were performed using GenStat Discovery (ver: 3.0) for windows (VSN International, 2008). Data were analysed using the completely randomized design (CRD). Differences between means were assessed using the least significant differences (LSD) at 5% probability levels. Relationships between variables were examined by simple linear correlation (*r*) and by regression analysis using Microsoft Excel (Schmuller, 2009).

## RESULTS

The different supplementations and water availability significantly affected shoot biomass of the seedlings (Figure 1). Water scarcity decreased the shoot biomass from 25.84 to 13.50 g, thus there was a 47% decrease in biomass after exposure to drought stress. In the irrigated conditions, there was a negative correlation between $Ca^{2+}$ and $K^+$ supplementation and shoot biomass of the seedlings (*r* = -0.453 and -0.835, respectively; Table 1). At the maximum $Ca^{2+}$ and $K^+$ concentration, there was a

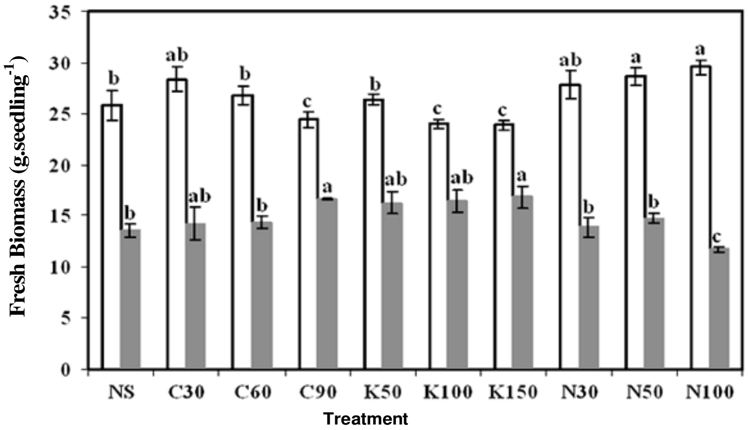

**Figure 1.** Fresh biomass of the *Brassica napus* L seedlings after different supplementations under control (white bars) and drought stress (grey bars) conditions. The vertical lines on the bars represent the standard deviation. NS, non-supplemented.

**Table 1.** Correlation coefficient of the different supplementations under irrigated and drought stress conditions with shoot biomass, tissue N and C content and C/N ratio

| Coefficient | Irrigated | | | | Drought | | | |
|---|---|---|---|---|---|---|---|---|
| | Biomass | N | C | C/N | Biomass | N | C | C/N |
| Ca | -0.453 | 0.988* | 0.889 | -0.897 | 0.903* | 0.974* | 0.994** | -0.749 |
| K | -0.835 | 0.935 | 0.800 | -0.963* | 0.861 | 0.914 | 0.887 | -0.924 |
| N | 0.974* | 0.856 | 0.999** | 0.534 | -0.462 | 0.988* | -0.817 | -0.995** |

* and ** indicate statistical significance at $P < 0.01$ and 0.05, respectively.

5 and 7% decrease in the shoot biomass compared with the non-supplemented control seedlings (Figure 1). However, under drought stress conditions, there was a positive correlation between $Ca^{2+}$ and $K^+$ application and shoot biomass (*r* = 0.903 and 0.861, respectively; Table 1). At the maximum $Ca^{2+}$ and $K^+$ application, the shoot biomass was 16.64 and 16.83 g under drought stress conditions, indicating 23 and 25% increase in biomass compared with the non- supplemented seedlings (Figure 1). The N supplementation, however, has a strong positive correlation with shoot biomass under irrigated conditions (*r* = 0.974; Table 1), but a negative correlation under drought stress conditions (*r* = -0.462; Table 1). There was 14% increase in the shoot biomass under irrigated conditions. However, under drought stress conditions, there was initially an increase, but at the maximum N concentration, there was 13% decrease in the shoot biomass.

The supplementation affected the shoot N content under both irrigated and drought stress conditions. N content of the dried shoot biomass under irrigated conditions was 3.38%, which decreased to 2.42% after exposure to drought stress (Figure 2). Thus, there was a 28% decrease in the shoot N content in drought stress conditions, compared with irrigated control. There was an increase in the N content of the tissue under both irrigated and drought stress condition after supplementation with $Ca^{2+}$, $K^+$ or N. Furthermore, there was a strong correlation between $Ca^{2+}$, $K^+$ and N supplementation and the shoot N content under both irrigated (*r* = 0.988. 0.935 and 0.856, respectively; Table 1) and drought stress conditions (*r* = 0.974, 0.914 and 0.988, respectively; Table 1). At the maximum $Ca^{2+}$ concentration, shoot N content was 3.58 and 2.78% under irrigated and drought stress conditions, respectively (Figure 2). Thus, there was 5.8 and 12.9% increase in tissue N content over the non- supplemented seedlings under irrigated and drought stress. $K^+$ application also enhanced tissue N content under both irrigated and drought stress conditions. There was a gradual increase in the tissue N content with increasing $K^+$ supplementation under both irrigated and drought stress conditions. The maximum tissue N content (3.62 and 2.79%) was obtained at 150 mM $KNO_3$ application under both the conditions (Figure 2). Similarly, there was a linear relationship between N supplementation and tissue N content and maximum tissue N content under both irrigated and drought stress condition (3.74 and 2.95%, respectively) was obtained after 100 mM $NH_4NO_3$ supplementation (Figure 2).

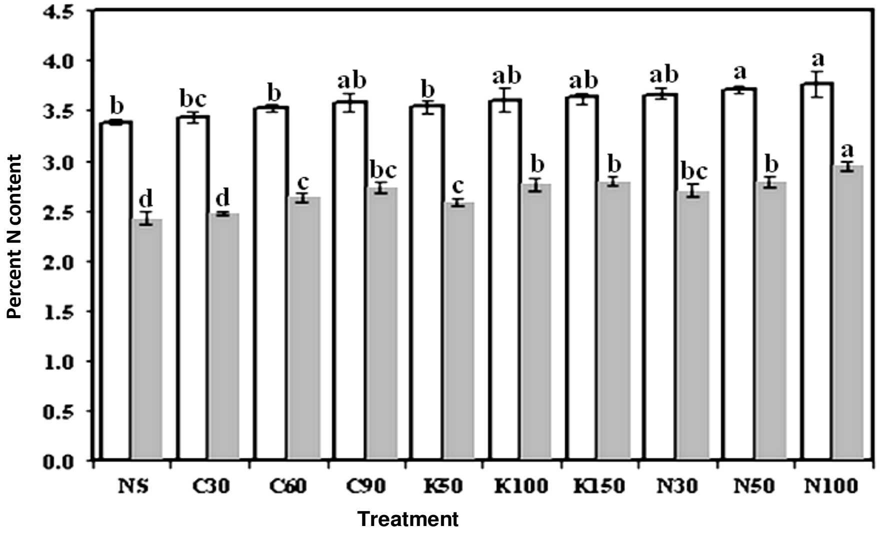

**Figure 2.** Nitrogen content of the above ground biomass of *Brassica napus* L seedlings after different supplementations under control (white bars) and drought stress (grey bars) conditions. Data is the mean ± SD of at least 9 seedlings. NS, non-supplemented.

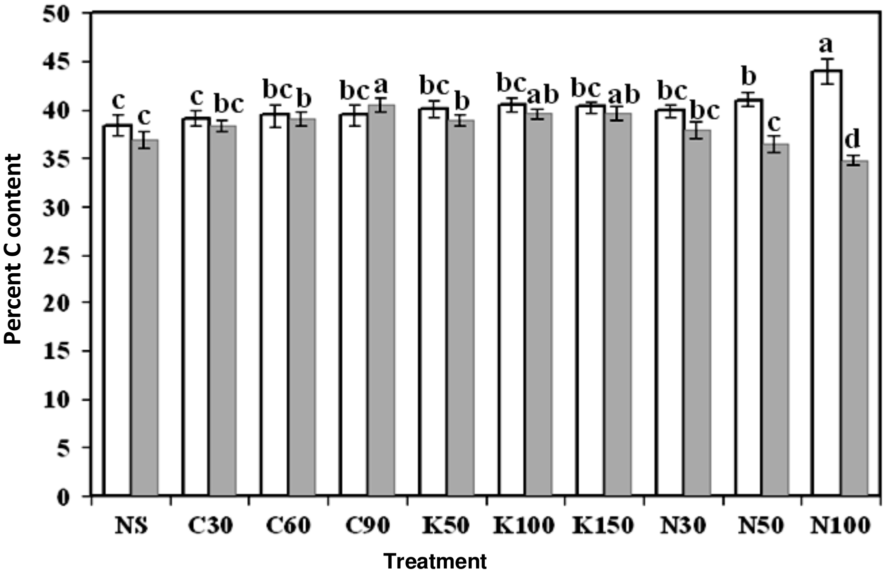

**Figure 3.** Carbon content of the above ground biomass of *Brassica napus* L seedlings after different supplementations under control (white bars) and drought stress (grey bars) conditions. Data is the mean ± SD of at least 9 seedlings. NS, Non-supplemented.

Similar to the tissue N content, the shoot C content was also affected by the different supplementations, as well as water application. The C content of the dried shoot biomass under irrigated conditions was 38.45%, which decreased to 36.87% after exposure to drought stress. Thus, compared with irrigated control, there was 4.11% decrease in the shoot C content in drought stress conditions (Figure 3). There was a slight increase in the C content of after supplementation with $Ca^{2+}$ and $K^+$, but N supplementation stimulated C accumulation only in irrigated conditions. Thus, there was a positive correlation between the tissue C content with $Ca^{2+}$, $K^+$ and N supplementation under irrigated conditions ($r$ = 0.889, 0.800 and 0.999, respectively; Table 1). When seedlings were exposed to drought stress conditions, there was a positive correlation between $Ca^{2+}$ and $K^+$ supplementation and C content ($r$ = 0.994 and 0.887, respectively; Table 1), but a negative correlation between

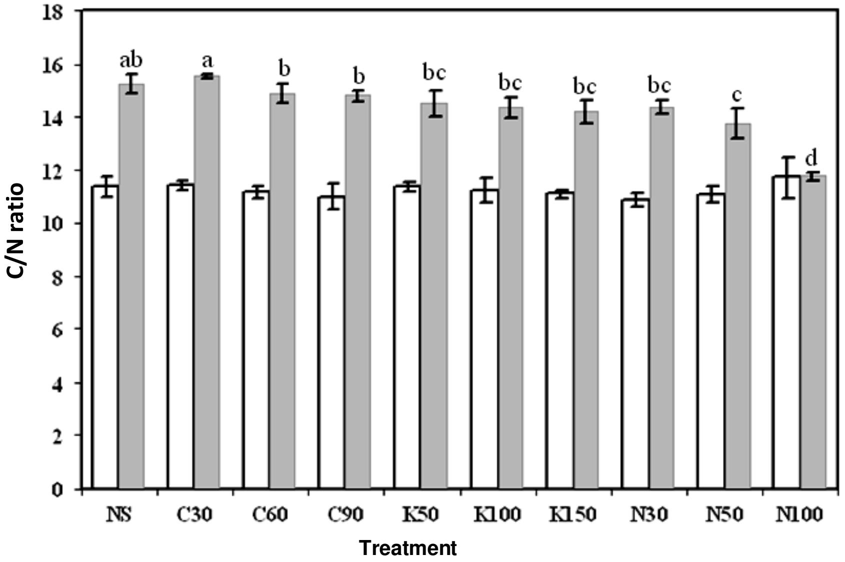

**Figure 4.** C/N ratio of the above ground biomass of *Brassica napus* L seedlings after different supplementations under control (white bars) and drought stress (grey bars) conditions. Data is the mean ± SD of at least 9 seedlings. NS, non-supplemented.

N supplementation and C content (*r* = -0.817, Table 1). At the maximum $Ca^{2+}$ concentration, shoot C content was 40.46% under drought stress conditions, thus there was 9.72% increase compared with the non supplemented seedlings under similar conditions (Figure 3). Similarly, maximum 150 mM $KNO_3$ supplementation also increased the tissue C content to 39.59% under drought stress conditions, thus showing 7.37% increase over non supplemented seedlings. Supplementing the seedlings with N under irrigated conditions increased the C content to 43.98%; however, the tissue C content was reduced to 37.73% of dry biomass when soil was amended with N before drought (Figure 3).

Water availability and supplementation with the $Ca^{2+}$, $K^+$ or N has variably affected the C/N ratio of the *B. napus* L. seedlings. Without supplementation, the C/N ratio increased from 11.37 to 15.22 (Figure 4). Similarly, there was a positive correlation between supplementing the seedlings with N and C/N ratio under irrigated conditions only (*r* = 0.534, Table 1). Under drought stress conditions, there was a negative correlation between the supplementations and C/N ratio (Table 1). At the maximum $Ca^{2+}$ supplementation, the C/N ratio decreased to 11.02 and 14.79, indicating 3.08 and 2.82% decrease over the non- supplemented seedlings under irrigated or drought stress conditions (Figure 4). Similarly, after the maximum $K^+$ supplementation, the tissue C/N ratio decreased to 11.10 and 14.18 (2.32 and 6.83% reduction respectively compared with the non supplemented seedlings). However, supplementation with N decreased tissue C/N ratio more significantly under both irrigated and drought stress conditions. At the maximum N supplementation, the tissue C/N ratio was 11.71 and 11.77, indicating 3.03% increase and 22.70% decrease over the non supplemented seedlings under similar conditions (Figure 4).

## DISCUSSION

The rate of transpiration, which is closely related with carbon assimilation through photosynthesis and nutrients uptake in plants, determines the biomass accumulation. Exposure to drought stress generally decreases biomass accumulation by inhibiting the rate of photosynthesis, as well as nutrients uptake (Flexas and Medrano, 2002; Aerts and Chapin, 2000). A significant reduction in fresh biomass of *B. napus* seedlings was observed after exposure to drought stress. Though the exogenous application of $Ca^{2+}$ and $K^+$ adversely affected the fresh biomass under irrigated conditions, there was an amelioration of the drought stress and a subsequent increase in the fresh biomass. The N supplementation, however, has positively affected shoot biomass under irrigated conditions, but has a negative effect under drought stress (Figure 1). Our results of enhancement of biomass accumulation under drought stress with $Ca^{2+}$ and $K^+$ application could be due to stabilization effect of these ions on plant cells. Adequate $K^+$ fertilization of crop plants may facilitate osmotic adjustment, which maintains turgor pressure at lower leaf water potentials and can improve the ability of plants to tolerate drought stress (Egilla et al., 2001; Mengel and Arneke, 1982). Similarly, besides direct effects as a structural basis for plant drought-resistance, $Ca^{2+}$ as a signaling molecule regulates many processes improving drought tolerance including activation of high affinity nutrient transporters (Ma et al., 2009; Shao et al., 2008). Nutrient availability for plant growth is an important factor influencing primary production (Aerts and Chapin, 2000). The improved metabolites availability and water relations in seedlings supplemented with $Ca^{2+}$ and $K^+$ application could have sustained osmotic potential and root growth resulting in higher nutrient acquisition from the soil. Furthermore, our results for low biomass accumulation in seedlings provided with exogenous N are also in agreement with the previous findings of reduction in root growth, increase in certain diseases and high water use associated with excessive use of N fertilizers under dry conditions (Heitholt, 1990).

Besides several physiological changes in plant cells, the lack of soil solution under drought stress reduces the uptake of different nutrients. N and C are important plant nutrients essential for plant growth and survival under environmental stress conditions (Rachmilevitch et al., 2006). There was generally a decreasing trend of N and C accumulation in aboveground biomass after exposure to drought stress (Figures 2 and 3). Enhancement of plant N uptake (Ineson et al., 1998), rather than increased N use efficiency (Maranville and Madhavan, 2002), resulted in stimulation of plant C accumulation under warming. In our case, supplementing the seedlings with $Ca^{2+}$and $K^+$ or N stimulated the N uptake under both irrigated and drought stress conditions (Figure 2). Rubisco contained the major proportion of leaf N thus the concentration of N directly determines C fixation activity. As a consequence of increase in N content with the addition of different supplements, an increase in the rate of C assimilation was expected. $Ca^{2+}$and $K^+$ had stimulated the C accumulation under both irrigated and drought stress conditions. The excitation energy derived from the electron transport (ET) in the photosynthetic apparatus is normally utilized for carbon assimilation (*An*). There is generally an increase in the ET/*An* in water stressed plants, suggesting partial diversion to the photosynthetic reduction of $O_2$ via photorespiration (Osório et al., 2006). The C metabolism in the cell depends on the assimilation through photosynthesis, consumption through respiration and allocation to different metabolic pools (Lamberts and Poorter, 1992). The higher C accumulation could be due to osmotic adjustment, decreased rate of respiration and maintenance of photosynthesis with the $Ca^{2+}$ or $K^+$ application (Egilla et al., 2001). Though the N content of the leaf dry matter was increased after supplementation with N under both irrigated and drought stress conditions, an increase in the C content was noted only in irrigated conditions (Figure 3). The increased damage suffered due to photo-oxidative stress with enhanced N supplementation could have decreased the rate of photosynthesis and increased demand for carbon in osmotic adjustment under drought stress conditions (Mozafar, 1993; Stitt et al., 2002; Guo et al., 2005). Furthermore, this lack of response of C accumulation could be due to N saturation under drought stress conditions.

Similarly, the tissue C/N ratio is also an important factor regulating the yield potential and an increase in the C/N ratio in the *B. napus* L. seedlings after exposure to drought stress was noted (Figure 4). As there was little effect of drought on C accumulation, the reduced uptake of N was the major reason for this increase. Supplementation of the seedlings with $Ca^{2+}$ and $K^+$ or N did not significantly change the C/N ratio under irrigated conditions. However, there was a significant decrease in the C/N ratio in seedlings supplemented with N under drought stress conditions. Drought adaptation requires a shift in ATP input into the energy-requiring processes towards greater expenses for ion transport and protein synthesis. The rate of respiration is increased to a greater extent after N supplementation to meet the energy requirement under drought stress (Zagdańska, 1995). The increased C content in seedlings supplemented with $Ca^{2+}$ and $K^+$ under drought stress indicated a protection of the cell resulting in the lower rate of respiration to meet the ATP requirement and consequently a better energy balance in these seedlings.

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