Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (\textit{Avena sativa} L cultivar Kent)

Mohammad Abass Ahanger*, R.M Agarwal, Nisha Singh Tomar and Madhup Shrivastava

School of Studies in Botany, Jiwaji University, Gwalior, MP, India

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Potassium is actively involved in many functions such as enzyme activation, osmotic adjustment and uptake of deleterious ions like Na. Present report analyses the effectiveness of different potassium salts on growth and certain components of nitrogen metabolism and antioxidant system in oat and their possible role in amelioration of water stress. Potassium induced enhancement in the activities of nitrate reductase and aminotransferases was evident indicating a positive role of potassium in nitrogen metabolism. Potassium supplementation enhanced activities of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase) and contents of total phenols and tannins, probably strengthening both the enzymatic as well as non enzymatic antioxidant system. Free amino acids, proline and free sugars also exhibited the same trend in treated plants ensuring better plant growth.

 keywords: Potassium; PEG-6000; \textit{Avena sativa} L; Nitrate reductase; Aminotransferases and Antioxidants

Introduction

Inadequate soil moisture and unbalanced supply of mineral nutrients result in impaired soil fertility and decreased global food production especially in arid and semi-arid regions. Water stress is one of major environmental constraints limiting crop productivity the world over (see Agarwal et al. 1999; Auras et al. 2002). About 25% of the world’s agricultural land is affected by water stress (Jajarmi 2009). Changes in global climate have worsened the situation even more (Cominelli & Tonelli 2010). Water stress affects yield by depressing both sink and source, depending on the timing and the severity of stress with respect to plant phenology. Plants respond differently to water deficiency during different growth stages. The generative phase and the beginning of flowering are most frequently the periods of the greatest sensitivity to water deficit (Blum 1996; Istanbulluoglu et al. 2009).

It is estimated that around 60% of cultivated soils have growth limiting problems associated with mineral nutrient deficiencies and toxicities (Cakmak 2002). Increasing evidence suggest that mineral nutrient status of plants plays a critical role in increasing plant resistance to environmental stress factors and is important in exploiting the genetic potential of crop plants.

Potassium (K) is the third important macronutrient (i.e. after nitrogen and phosphorus) required for plant growth and is involved in many biochemical and physiological processes such as osmoregulation, stomatal movement, activation of enzymes, protein synthesis, photosynthesis, phloem loading and transport and in reducing excess uptake of ions such as Na and Fe in saline soils (Wang et al. 2013; Zorb et al. 2014).

Greater concentrations of potassium are found in young developing tissues and reproductive organs indicating its requirements in cell metabolism and growth. Potassium fertilizers are not subjected to leaching or volatilization and therefore can be applied to a wide range of environmental conditions by different methods of application. Availability of potassium to plants is highly variable and is mostly affected by root soil interaction (Ashley et al. 2006). In long distance transport, potassium is the dominant cation within the xylem and phloem sap neutralizing inorganic and organic anions, conferring high potassium mobility throughout the entire plant (Jeschke et al. 1997). Uptake and accumulation of potassium by plant cells is the primary driving force for their osmotic expansion.

Reactive oxygen species (ROS) affect normal metabolic functioning of plants because of effects on lipids, proteins as well as nucleic acids (Ahmad et al. 2010). Several defence mechanisms help plants to avert ROS induced alterations. Enzymatic and non enzymatic antioxidants present within the plant system mediate scavenging of ROS thereby preventing their accumulation and protecting plant cells from deleterious effects.

Oat (\textit{Avena sativa} L) is grown throughout the temperate zone as rabi crop and has a low summer heat requirement and greater tolerance of rain than other cereals, such as wheat, rye or barley, and therefore are particularly important in areas with cool, wet summers. Oat is an annual plant and can be planted either in autumn (for late summer harvest) or in the spring (for early autumn harvest). Its consumption is believed to lower low density lipoprotein (bad cholesterol) and it contains a wide range of functional ingredients having antioxidant property which are mostly concentrated in different parts of the kernel (Gray et al. 2000, Peterson...
Material and methods

Treatments and cultivation conditions

Seeds of *A. sativa* L. (oat) cultivar ‘KENT’ were obtained from Indian Grassland and Fodder Research Institute, Jhansi UP. Healthy seeds were selected and used for germination and growth under different treatments. Seeds were surface sterilized using 0.01% mercuric chloride and washed with distilled water. Each Petri plate was lined with Whatman filter paper number 1 in two layers and ten seeds were placed in each Petri plate. Three salts of potassium, that is, K2O, KCl and KNO3 were used in varying concentrations [0.1, 0.01 and 0.001 M (w/v)] and Polyethylene glycol (PEG-6000) was employed to induce water stress and water potential of solution was maintained at ~2.7 bars following Michel et al. (1983). Potassium salts were also used in treatments subjected to polyethylene glycol-6000 – induced water stress to comprehend possible role of potassium salts in ameliorating PEG-6000 induced water stress, if any. Petriplates were kept on culture racks under constant light and dark periods of 10/14 h. Seedlings were put to analysis seven days after sowing (7 DAS).

Pot experiments: Separate bottom perforated pots were maintained for different treatments in the botanical garden of School of Studies in Botany, Jiwaji University, Gwalior MP, India under natural conditions with day and night temperatures of 25/8 ± 4°C and relative humidity of 85/55 ± 10%. Each of the pots used was 24" × 12" × 10" in dimensions and were filled with well ploughed soil having the characteristics given in Table 1. Nitrogen (in the form of urea) and phosphorous (single super phosphate) fertilizers were applied as per the recommended requirement, that is, 120 and 30 kg ha−1 respectively. Two potassium salts K2O and KNO3 were applied at the rate of 0, 20, 40 and 60 kg ha−1. Replicates receiving only nitrogen and phosphorous but no potassium (0 kg ha−1) served as control. Equal number of seeds were sown at the depth of 3–4 cm. Growth of plants was monitored regularly. After 55 days leaves, roots and stem were separated for measurement of shoot/root length and fresh and dry weight.

Relative water content: Relative water content (RWC) of leaf was measured in fully expanded leaves following Weatherly’s (1950) method. Twenty discs (0.5 cm in diameter) were excised from the leaves (excluding mid vein) by cork borer and their fresh weight (FW) was determined. These discs were floated on distilled water in petri dishes/cavity discs for an hour to gain turgidity and thereafter removed and soaked on filter paper and then reweighed (TW). The samples were dried at 60°C for 24 h and then their dry weight (DW) was determined. RWC was calculated using the following formula:

\[
\text{RWC} (%) = \frac{(\text{FW} - \text{DW})}{\text{TW} - \text{DW}} \times 100
\]

Table 2. Shoot/root length and fresh/dry weight of oat (*A. sativa* L. cultivar KENT) seedlings raised under different potassium treatments (7 DAS).

| Treatments | Shoot length (cm) | Root length (cm) | Shoot fresh weight (mg) | Shoot dry weight (mg) | Root fresh weight (mg) | Root dry weight (mg) |
|------------|------------------|-----------------|-------------------------|-----------------------|------------------------|----------------------|
| Control    | 7.34 ± 0.51c     | 5.65 ± 0.44d    | 203.2 ± 1.9e             | 19.1 ± 0.035f         | 103.4 ± 1.5d           | 7.2 ± 0.21h           |
| 0.1 M KNO3 | 3.77 ± 0.67d     | 4.07 ± 0.60b    | 224.9 ± 2.4d             | 13.9 ± 0.21e          | 116.7 ± 1.2e           | 8.6 ± 0.17b           |
| 0.01 M KNO3| 7.98 ± 0.35b     | 6.21 ± 0.43b    | 301.5 ± 3.4b             | 27.2 ± 0.044f         | 116.0 ± 1.5e           | 8.9 ± 0.31b           |
| 0.001 M KNO3| 8.71 ± 0.66a     | 7.17 ± 0.57a    | 346.0 ± 4.0g             | 22.4 ± 0.042b         | 149.8 ± 2.5b           | 7.5 ± 0.23b           |
| 0.1 M K2O  | 2.42 ± 0.26d     | 2.97 ± 0.21f    | 96.3 ± 1.3f              | 10.5 ± 0.12d          | 69.4 ± 1.6g            | 6.7 ± 0.23h           |
| 0.01 M K2O | 8.18 ± 0.66a     | 6.65 ± 0.25b    | 316.7 ± 4.3b             | 27.9 ± 0.058e         | 159.6 ± 2.9b           | 10.9 ± 0.09h          |
| 0.001 M K2O| 7.73 ± 0.16b     | 6.38 ± 0.34b    | 246.8 ± 3.6c             | 21.1 ± 0.32b          | 106.0 ± 1.9d           | 8.7 ± 0.21h           |
| 0.1 M KCl  | 3.03 ± 0.58d     | 3.20 ± 0.68b    | 137.0 ± 1.8e             | 22.7 ± 0.21b          | 133.4 ± 2.5b           | 9.3 ± 0.14h           |
| 0.01 M KCl | 8.41 ± 0.71a     | 5.84 ± 0.23b    | 318.8 ± 2.3b             | 24.3 ± 0.26b          | 236.2 ± 2.3a           | 10.4 ± 0.21h          |
| 0.001 M KCl| 7.20 ± 0.27b     | 6.11 ± 0.11c    | 229.4 ± 3.0d             | 28.1 ± 0.07f          | 109.4 ± 3.7e           | 10.8 ± 0.25g          |

Note: Data followed by same letter are not significantly different at p < .05.
Estimation of Chlorophylls and Carotenoids: Chlorophyll contents in leaves were determined following Arnon’s method (1949). After extraction with 80% acetone final volume was made up to 5 ml with 80% acetone (v/v) and optical density was recorded at 480, 645 and 663 nm.

Lipid peroxidation: Lipid peroxidation was determined by measuring content of malondialdehyde (MDA) formation. For calculating MDA content an extinction coefficient (ε) of 155 mM$^{-1}$cm$^{-1}$ was used (Heath & Packer 1968).

Proline: Free proline was estimated following Bates et al. (1973). Dry plant material was extracted in sulphosalicylic acid [3% (w/v)] followed by centrifugation at 3000 g for 10 min. Two milliliter supernatant was mixed with glacial acetic acid and acid ninhydrin followed by incubation for 1 h at 100°C. The reaction was terminated in ice. Free proline was separated using toluene in a separating funnel and optical density was recorded at 520 nm.

Free amino acids: Free amino acids were estimated following the method outlined in Sadasivam and Manickam (2004). Powdered plant sample was extracted in 80% ethanol (v/v) and centrifuged at 2000 g for 20 min. Hundred microliter supernatant was made up to 2 ml with distilled water and to this 1 ml ninhydrin [prepared in 0.2 M citrate buffer (pH 5.0) and 2-methoxyethanol] was added and after incubating for 30 min 5 ml diluent was added. After 20 min optical density was recorded at 570 nm. Computation was done using standards.

Free sugars: Total free sugars were worked out using anthrone method. One hundred milligram powdered sample was extracted in ethanol (80%) followed by centrifugation at 5000 g for 10 min. About 0.5 ml supernatant was taken and volume was made up to 1 ml HCl (1N) and kept in water bath at boiling temperature. Thereafter, 4 ml anthrone (0.2%) was added and again kept in water bath for 10 min and optical density was recorded at 620 nm (Fong et al. 1953; Jain & Guruprasad 1989).

Phenols: 0.5 gm powdered plant material was extracted in 80% ethanol and centrifuged at 10,000 g for 20 min. The supernatant was collected and residue...
KNO₃ and 0.5% n-propanol] kept in dark for 3 h at 30°C.

Phosphate buffer maintained at pH 7.5 containing 200 mM in 5 ml tubes.

Microgram fresh plant sample was cut into small pieces adopted by Sharma and Agarwal (2002). Three hundred.

1.6.6.1) assay was done following Srivastava (1974) as tannic acid (Swain & Hills 1959).

Control 0.102 ± 0.007 c 0.046 ± 0.003 c 0.149 ± 0.010d 0.101 ± 0.006d

Treatments Chlorophyll a Chlorophyll b Total Chlorophylls Carotenoids

Potassium treatments (7 DAS).

Control 0.227 ± 0.004 a 0.094 ± 0.013a 0.321 ± 0.007a 0.251 ± 0.009a

Treatments Chlorophyll a Chlorophyll b Total Chlorophylls Carotenoids

Potassium treatments and PEG-induced water stress (7 DAS).

Note: Data followed by same letter are not significantly different at p < .05.

Table 5. Chlorophyll and Carotenoid (mg g⁻¹ fresh wt) contents in oat (A. sativa L. cultivar KENT) seedlings raised under different potassium treatments (7 DAS).

| Treatments          | Chlorophyll a | Chlorophyll b | Total Chlorophylls | Carotenoids |
|---------------------|---------------|---------------|--------------------|-------------|
| Control             | 0.227 ± 0.004a| 0.094 ± 0.013a| 0.321 ± 0.007a     | 0.251 ± 0.009a|
| −2.7 bars           | 0.124 ± 0.007a| 0.054 ± 0.014a| 0.178 ± 0.006a     | 0.138 ± 0.011f|
| −2.7 bars + 0.1 M KNO₃ | 0.213 ± 0.015b| 0.075 ± 0.019c| 0.278 ± 0.021c     | 0.241 ± 0.018b|
| −2.7 bars + 0.01 M KNO₃ | 0.217 ± 0.005b| 0.082 ± 0.007b| 0.291 ± 0.015b     | 0.236 ± 0.016bc|
| −2.7 bars + 0.1 M K₂O | 0.205 ± 0.013a| 0.074 ± 0.011a| 0.282 ± 0.019bc    | 0.186 ± 0.007c|
| −2.7 bars + 0.01 M K₂O | 0.195 ± 0.013a| 0.071 ± 0.004d| 0.166 ± 0.015f     | 0.235 ± 0.011c|
| −2.7 bars + 0.1 M KCl | 0.210 ± 0.010bc| 0.084 ± 0.018b| 0.295 ± 0.005b     | 0.225 ± 0.020c|
| −2.7 bars + 0.01 M KCl | 0.193 ± 0.012cd| 0.069 ± 0.012b| 0.223 ± 0.001d     | 0.203 ± 0.010d|

Note: Data followed by same letter are not significantly different at p < .05.

Table 6. Chlorophyll and Carotenoid (mg g⁻¹ fresh wt.) contents in oat (A. sativa L. cultivar KENT) seedlings raised under different potassium treatments and PEG-induced water stress (7 DAS).

| Treatments          | Chlorophyll a | Chlorophyll b | Total Chlorophylls | Carotenoids |
|---------------------|---------------|---------------|--------------------|-------------|
| Control             | 0.227 ± 0.004a| 0.094 ± 0.013a| 0.321 ± 0.007a     | 0.251 ± 0.009a|
| −2.7 bars           | 0.124 ± 0.007a| 0.054 ± 0.014a| 0.178 ± 0.006a     | 0.138 ± 0.011f|
| −2.7 bars + 0.1 M KNO₃ | 0.213 ± 0.015b| 0.075 ± 0.019c| 0.278 ± 0.021c     | 0.241 ± 0.018b|
| −2.7 bars + 0.01 M KNO₃ | 0.217 ± 0.005b| 0.082 ± 0.007b| 0.291 ± 0.015b     | 0.236 ± 0.016bc|
| −2.7 bars + 0.1 M K₂O | 0.205 ± 0.013a| 0.074 ± 0.011a| 0.282 ± 0.019bc    | 0.186 ± 0.007c|
| −2.7 bars + 0.01 M K₂O | 0.195 ± 0.013a| 0.071 ± 0.004d| 0.166 ± 0.015f     | 0.235 ± 0.011c|
| −2.7 bars + 0.1 M KCl | 0.210 ± 0.010bc| 0.084 ± 0.018b| 0.295 ± 0.005b     | 0.225 ± 0.020c|
| −2.7 bars + 0.01 M KCl | 0.193 ± 0.012cd| 0.069 ± 0.012b| 0.223 ± 0.001d     | 0.203 ± 0.010d|

Note: Data followed by same letter are not significantly different at p < .05.

was re-extracted. The supernatant was evaporated to dryness and dissolved in 5 ml distilled water. Aliquot (0.1 ml) was taken and made up to 2 ml with distilled water. One normality Folin-Ciocalteu’s reagent was added and incubated for 3 min. Twenty percent Na₂CO₃ (2 ml) was added and kept in boiling water bath for 1 min and subsequently cooled and read at 650 nm (Malick & Singh 1980).

Tannins: Dry powdered plant material was extracted in distilled water and heated for 30 min in boiling water bath followed by centrifugation at 2000 g for 20 min. The supernatant was collected and final volume was made up to 10 ml with distilled water. Hundred µL extract was taken and made up to 2 ml with distilled water followed by addition of Folin Denis reagent (1 ml) and 35% (w/v) Na₂CO₃ (2 ml) and thereafter kept in dark for 45 min at room temperature. Optical density was recorded at 700 nm and computation was done using standards and expressed as mg g⁻¹ dry weight tannic acid (Swain & Hills 1959).

Nitrate reductase assay: Nitrate reductase (EC 1.6.6.1) assay was done following Srivastava (1974) as adopted by Sharma and Agarwal (2002). Three hundred microgram fresh plant sample was cut into small pieces in 5 ml tubes filled with incubation medium [0.1 M phosphate buffer maintained at pH 7.5 containing 200 mM KNO₃ and 0.5% n-propanol] kept in dark for 3 h at 30°C. Thereafter, 1 ml aliquot was taken and 1 ml sulfanilamide (prepared in 1 N HCL) and 1 ml 0.2% 1-naphthyl ethylene diamine dihydrochloride were added to it followed by thorough mixing and left for 25 min to develop color. Optical density was recorded at 540 nm.

Assay of Aminotransferases: Aspartate (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) activity was determined following Reitman and Frankel’s (1957) method. One hundred milligram sample was homogenized in 1.5 ml chilled sodium potassium buffer (pH 7). After centrifugation for 15 min at 4000 g at 4°C the supernatant was used as enzyme source. 50 µl supernatant was added to 0.1 ml substrate buffer (100 mM phosphate buffer containing 2-oxoglutarate (2 mM) and 100 mM L-aspartate or 200 mM DL-alanine) followed by thorough mixing and incubation at 37°C in a water bath (incubation time for aspartase is 60 min and for alanine 30 min). Thereafter, 0.1 ml DNPH (2, 4-dinitrophenyl hydrazine prepared in 1 M HCL) was added and after 20 min 1 ml sodium hydroxide (0.4 M) was added and mixed. After 5 min optical density was recorded at 540 nm.

For superoxide dismutase (SOD) and catalase (CAT) fresh plant sample (0.1 gm) was homogenized in chilled potassium phosphate buffer (pH 7.5) containing 0.1% PVP and 0.5 mM EDTA followed by centrifugation at 12,000 g for 15 min and the supernatant was used as enzyme source. However, for ascorbate
peroxidase (APX) crushing buffer contained 2 mM ascorbate. Protein content was estimated as per Lowry et al. (1951).

**Superoxide Dismutase:** SOD (EC 1.15.1.1) activity was determined following the method of Dhindsa et al. (1981). The assay mixture consisting of 1.5 ml potassium phosphate buffer (pH 7.8), 0.2 ml methionine, 0.1 ml enzyme extract with equal volume of 1 M Na₂CO₃, 2.25 mM nitroblue tetrazolium, 3 mM EDTA, riboflavin and 1 ml double distilled water was incubated under 15 W fluorescent lamp for 15 min. Blank A containing the same reaction mixture was placed in the dark. Blank B containing the same reaction mixture except the enzyme extract was placed in light along with the sample. The reaction was terminated by switching off the light and the tubes were covered with a black cloth. Absorbance of each sample along with blank B was read against blank A at 560 nm and the difference in percent color reduction between blank B and the sample was calculated. Fifty percent color reduction was considered as one unit of enzyme activity and activity was expressed as EU mg⁻¹ protein.

**Catalase:** The activity of CAT (EC 1.11.1.6) was assayed in accordance with Aebi (1984). Assay mixture contained 50 mM potassium phosphate buffer (pH 7), 100 mM H₂O₂ and 100 µL enzyme extract in a final volume of 2 ml. Change in optical density at 240 nm was recorded for 2 min. Activity was calculated using extinction coefficient (ε) 0.036 mM⁻¹ cm⁻¹ and was expressed as enzyme unit mg⁻¹ protein.

**Ascorbate Peroxidase:** APX (EC 1.11.1.1) was spectrophotometrically assayed following Nakano and Asada (1981). The assay mixture containing 25 mM potassium phosphate buffer (pH 7.0), 100 mM H₂O₂ and 1 ml enzyme extract in a final volume of 1 ml was incubated for 15 min under 15 W fluorescent lamp. The absorbance was recorded at 290 nm against blank A at 290 nm.

### Table 7. Chlorophyll a, b, Total Chlorophylls and Carotenoid (mg g⁻¹ fr wt.) contents in flag leaf of *A. sativa* L. cultivar KENT supplied with different salts of potassium (55 DAS).

| Treatments      | Chlorophyll a | Chlorophyll b | Total Chlorophylls | Carotenoids |
|-----------------|---------------|---------------|--------------------|-------------|
| Control         | 0.4538 ± 0.022d | 0.3633 ± 0.030d | 0.8175 ± 0.052c   | 0.7734 ± 0.055c |
| K₂O 20 kg ha⁻¹  | 0.5365 ± 0.014c | 0.7315 ± 0.059a | 1.0653 ± 0.186a   | 0.9586 ± 0.041b |
| K₂O 40 kg ha⁻¹  | 0.5927 ± 0.015ab| 0.5603 ± 0.017b | 1.1540 ± 0.023a   | 1.1143 ± 0.027b |
| K₂O 60 kg ha⁻¹  | 0.5766 ± 0.047b | 0.5183 ± 0.052bc| 1.1258 ± 0.097a   | 1.129 ± 0.093b  |
| KNO₃ 20 kg ha⁻¹ | 0.5816 ± 0.008b | 0.5841 ± 0.098b | 1.1306 ± 0.121a   | 0.9527 ± 0.037b |
| KNO₃ 40 kg ha⁻¹ | 0.5700 ± 0.012b | 0.5153 ± 0.024bc| 1.0001 ± 0.091a   | 1.1229 ± 0.014a |
| KNO₃ 60 kg ha⁻¹ | 0.6300 ± 0.007a | 0.5540 ± 0.008b | 1.1865 ± 0.008a   | 1.1627 ± 0.034a |

Note: Data followed by same letter are not significantly different at p < .05.
Asada (1981). 3 ml assay mixture contained 50 mM potassium phosphate buffer (pH 6), 0.1 mM EDTA, 0.5 mM ascorbate and 100 µL H$_2$O$_2$ and 100 µL enzyme extract. H$_2$O$_2$ dependent oxidation of ascorbate was followed by change in the absorbance at 290 nm. APX activity was calculated using extinction coefficient (ε) 2.8 mM$^{-1}$ cm$^{-1}$ and expressed as enzyme unit mg$^{-1}$ protein.

![Figure 2. Nitrate reductase activity (µ mole NO$_2$ produced hr$^{-1}$ g$^{-1}$ fr wt) in flag leaf of A. sativa L. cultivar KENT supplied with different salts of potassium (55 DAS). Note: Data followed by same letter are not significantly different at p < .05.](image)

![Figure 3. Alanine (A) and Aspartate (B) aminotransferase activity (µ mol keto acid released mg$^{-1}$ protein min$^{-1}$) in oat (A. sativa L. cultivar KENT) seedlings raised under different potassium treatments (7 DAS). Note: Data followed by same letter are not significantly different at p < .05.](image)
Estimation of K, Na and Ca

Dry plant samples were digested in triacid (H$_2$SO$_4$ + HNO$_3$ + HClO$_4$ in 9:3:1 ratio) and the volume of white colorless digested material was made up to 100 ml with distilled water and was subsequently filtered. Filtrate was read directly on digital flame photometer using K, Na and Ca filters separately (Tomar & Agarwal 2013; Jatav et al. 2014).

Statistical analysis

Data presented are mean of four replicates with standard error (±SE) calculated. All the data were statistically analyzed by analysis of variance with the MSTAT C PROGRAM (Mich. University, East Lasing Mich. USA). Least significant difference (LSD) was calculated for the significant data at $p < .05$. Data followed by same letter are not significantly different by LSD test at $p < .05$.

Results and discussion

Increase in shoot/root length and fresh/dry weight was observed as a result of potassium application excepting for treatments using 0.1 M concentration of potassium salts in which a decline was observed, may be because of osmotic stress (Table 2). Singh et al. (2000) and Jatav et al. (2012) have also found better growth and yield of
wheat crop with the addition of potassium. Increase in root and shoot length as a result of application of potassium has been earlier reported in *Triticum aestivum* L. by Shirazi et al. (2005) also. KNO₃ induced increase in fresh and dry weight has earlier been reported in sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) by Jabeen and Ahmad (2011) as well.

Water stress induced using polyethylene glycol (PEG 6000) caused reduction in shoot/root length and fresh / dry weight, however, this effect was ameliorated by application of potassium to some extent (Table 3). PEG-induced reduction in seedling growth has been reported in *Zea mays* L (Farsiani & Ghobadi 2009) and *Triticum aestivum* L (Jatav et al. 2012). This reduction in plant

Table 8. Phenols and Tannins (mg g⁻¹ dry wt.) contents in *A. sativa* L. cultivar KENT supplied with different salts of potassium (55 DAS).

| Treatments        | Phenols | Tannins |
|-------------------|---------|---------|
|                   | Flag leaf | Stem | Root | Flag leaf | Stem | Root |
| Control           | 3.93 ± 0.077ᵃ | 3.72 ± 0.110ᵇ | 1.07 ± 0.047ᵇ | 77.00 ± 1.22ᵇ | 22.52 ± 0.27ᵈ | 21.22 ± 0.72ᵈ |
| K₂O 20 kg ha⁻¹    | 4.18 ± 0.031ᵇ | 3.40 ± 0.212ᵈ | 1.16 ± 0.019ᵇ | 79.02 ± 1.73ᵇ | 24.75 ± 0.75ᵈ | 22.37 ± 0.25ᵈ |
| K₂O 40 kg ha⁻¹    | 4.64 ± 0.050ᵇ | 4.80 ± 0.070ᵇ | 1.44 ± 0.021ᵇ | 82.32 ± 1.28ᵇ | 34.77 ± 0.29ᵇ | 25.52 ± 0.84ᵇ |
| K₂O 60 kg ha⁻¹    | 5.78 ± 0.031ᵃᵇ | 5.92 ± 0.047ᵃ | 1.87 ± 0.025ᵃ | 88.95 ± 1.47ᵇ | 36.25 ± 0.47ᵇ | 28.25 ± 0.85ᵇ |
| KNO₃ 20 kg ha⁻¹   | 4.19 ± 0.052ᵇ | 4.07 ± 0.085ᵇ | 1.42 ± 0.026ᵇ | 75.27 ± 1.33ᵇ | 37.65 ± 1.11ᵇ | 25.65 ± 0.96ᵇ |
| KNO₃ 40 kg ha⁻¹   | 5.02 ± 0.026ᵇ | 4.95 ± 0.210ᵇ | 1.49 ± 0.013ᵇ | 87.30 ± 0.47ᵇ | 39.22 ± 0.44ᵇ | 30.62 ± 0.23ᵇ |
| KNO₃ 60 kg ha⁻¹   | 6.39 ± 0.020ᵃᵇ | 5.97 ± 0.062ᵃ | 2.15 ± 0.050ᵃ | 94.20 ± 1.79ᵇ | 47.27 ± 0.93ᵃ | 33.75 ± 0.62ᵃ |

Note: Data followed by same letter are not significantly different at *p* < .05.
height is attributed to a decline in the cell enlargement and
greater leaf senescence in the plants subjected to water
stress (Manivannan et al. 2007b). Added potassium has
been reported to alleviate the negative effects of water
stress in *Oryza sativa* L (Tiwari et al. 1998), sorghum,
mustard and groundnut (Umar 2006) and *Triticum aesti-
vum* L (Jatav et al. 2012) as well.

Moreover, increase in number of tillers per pot as a
result of potassium supplementation was obvious at the
later stage of development with slight increase in RWC
(Table 4) indicating positive role of potassium in
hydration and thereby in the maintenance of turgor and
subsequently growth as well. Other reports of improved
water use efficiency as a result of potassium application
are also available such as in *O. sativa* L (Tiwari et al.
1998), *Cicer arietinum* L (Ali et al. 2005), sorghum,
mustard and groundnut (Umar 2006), *Brassica napus* L
(Fanaei et al. 2009) and *Triticum aestivum* L (Jatav
et al. 2012).

An increase in Chlorophyll and carotenoid contents
was also found as a result of potassium treatments
excepting for higher concentration, that is, 0.1 M.
Reduction in chlorophyll and carotenoid contents
resulted due to polyethylene glycol 6000 induced water
stress was overcome substantially as indicated in the
treatments in which potassium salts were applied along-
with PEG-6000 (Tables 5–7). Loss of chlorophyll con-
tents under water stress is considered to be the main
cause of inactivation of photosynthesis. Water deficit
induced reduction in chlorophyll contents is attributed
to the loss of chloroplast membranes, excessive swelling,
distortion of the lamellae vesiculation and the appearance
of lipid droplets (Kaiser et al. 1981). Exposure to
environmental stresses results in inhibition of chlorophyll

![Figure 6. Superoxide dismutase (A), Catalase (B) and Ascorbate peroxidase (C) (EU mg⁻¹ protein) activity in flag leaf of *A. sativa*
L. cultivar KENT supplied with different salts of potassium (55 DAS).](image)

Note: Data followed by same letter are not significantly different at *p* < .05.
biosynthesis (Khan 2003) as well as instability of pigment protein complex.

Nitrate reductase activity in both shoot and root showed an increase in seedlings raised with potassium supplementation and increase in activity was greater where potassium was used as potassium nitrate (Figure 1(a) and Figure 2). Polyethylene glycol-6000 induced stress reduced nitrate reductase activity in oat seedlings, and the negative effect of stress was ameliorated to some extent by application of potassium (Figure 1(b)). Reduction due to polyethylene glycol-6000 induced water stress in rice nitrate reductase activity has been reported by Pandey and Agarwal (1998). An increase in nitrate reductase activity with the application of potassium has earlier been reported in *Cicer arietinum* L (Sharma & Agarwal 2002; Ali et al. 2005), *Lepidium sativum* L (Dhawan et al. 2011), sunflower and safflower (Jabeen & Ahmad 2011) as well.

Potassium supplementation caused considerable increase in alanine and aspartate aminotransferase activity in the shoot as well as root (Figure 3(a) and 3(b)). PEG-6000 induced water stress has resulted in reduction of alanine and aspartate aminotransferase activity. Treatment of potassium has however, alleviated this effect to some extent (Figure 4(a) and 4(b)). Similar trend was obvious for aminotransferases under field conditions (Figure 5(a) and 5(B)). Osmotic and water stress induced reduction in aspartate and alanine aminotransferase activity along with altered amino acid contents has been reported in rice (Pandey et al. 2004). Aminotransferases serve as a link between carbohydrate and amino acid metabolism. Increased activity of aminotransferases leads to greater synthesis of certain amino acids which may play some protective role under stress conditions (Rao & Ramamoorthy 1981). Besides, having key role in primary nitrogen assimilation, aminotransferases also help in transfer of reducing equivalents and bringing about interchanges of carbon and nitrogen pools within subcellular compartments (Torre et al. 2007).

Table 9. Free amino acids (mg g⁻¹ dry wt.), Proline (µ mole proline g⁻¹ dry wt.) and Free sugars (mg g⁻¹ dry wt.) contents in *A. sativa* L. cultivar KENT supplied with different salts of potassium (55 DAS).

| Treatments       | Flag leaf | Stem | Root | Flag leaf | Stem | Root | Flag leaf | Stem | Root |
|------------------|-----------|------|------|-----------|------|------|-----------|------|------|
| Control          | 70.32 ± 0.86c | 3.72 ± 0.46c | 2.97 ± 0.34c | 3.72 ± 0.46c | 3.72 ± 0.46c | 2.97 ± 0.34c | 3.72 ± 0.46c | 3.72 ± 0.46c | 2.97 ± 0.34c |
| K₂O₂ 0 kg ha⁻¹   | 72.25 ± 1.11c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c | 4.35 ± 0.34c |
| K₂O₄ 0 kg ha⁻¹   | 92.72 ± 1.89c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c | 7.50 ± 0.69c |
| K₂O₆ 0 kg ha⁻¹   | 138.12 ± 1.8a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a | 8.72 ± 0.33a |
| KNO₃ 20 kg ha⁻¹  | 73.15 ± 0.35c | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab | 7.80 ± 0.33ab |
| KNO₃ 40 kg ha⁻¹  | 137.27 ± 3.3a | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b | 8.07 ± 0.31b |
| KNO₃ 60 kg ha⁻¹  | 101.42 ± 1.4b | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a | 11.32 ± 0.31a |

Note: Data followed by same letter are not significantly different at p < 0.05.
increase in *O. sativa* L. (Yu-Chuan et al. 2008) and *Panax ginseng* (Devi et al. 2012) also.

Osmotic constituents like free amino acids, free proline and free sugars also increased in plants receiving potassium supplementation which may improve their water/salinity stress tolerance by possible enhancement of osmotic adjustment (Table 9). Accumulation of compatible osmotic constituents is one of the important and basic adaptation strategies for better growth and adaptation under environmental stresses (Chen et al. 2007). Proline is one the important organic osmolytes utilized in osmotic adjustment leading to maintenance of turgor/osmotic balance, through better extraction of water from the soil (Kusaka et al. 2005; Gonzalez et al. 2010). Proline acts as metal chelator, antioxidative defense molecule bringing concentrations of ROS within normal ranges, thus preventing oxidative burst (Hayat et al. 2012).

Malonaldehyde content, a product of membrane lipid peroxidation, decreased in plants supplied with potassium salts possibly indicating stability to membranes and hence reducing leakage (Table 10). Lipid peroxidation, the oxidative deterioration of unsaturated lipids, is an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form deleterious compounds including reactive carbonyl compounds. Decrease in MDA content as a result of potassium supplementation have been reported in *O. sativa* L. (Yu-Chuan et al. 2008) and *Hordeum vulgare* L. (Hafsi et al. 2010). Various kinds of antioxidants with different functions inhibit lipid peroxidation and the deleterious effects caused by the lipid peroxidation products (El-Beltagi & Mohamed 2013). Present experiments provide evidence that potassium treated plants are equipped with improved antioxidant system that includes increased antioxidant contents and enhancement in the activity of SOD, CAT and APX.

Increased uptake of potassium and calcium was obvious in all potassium treatments and in addition potassium supplementation reduced the uptake and accumulation of sodium ions (Table 11). Sodium shows antagonistic behavior towards potassium. Higher potassium content observed in leaf indicates efficient uptake of potassium and selectivity of plants towards potassium uptake in comparison to sodium. Increased uptake of

### Table 10. Malondialdehyde content (µ mol g⁻¹ fr. wt.) in flag leaf of *A. sativa* L. cultivar KENT supplied with different salts of potassium (55 DAS).

| Treatments | MDA content  |
|------------|--------------|
| Control    | 1.0760 ± 0.054a |
| K₂O 20 kg ha⁻¹ | 0.8590 ± 0.035b |
| K₂O 40 kg ha⁻¹ | 0.8236 ± 0.025c |
| K₂O 60 kg ha⁻¹ | 0.7173 ± 0.010d |
| KNO₃ 20 kg ha⁻¹ | 0.8813 ± 0.008b |
| KNO₃ 40 kg ha⁻¹ | 0.8613 ± 0.082b |
| KNO₃ 60 kg ha⁻¹ | 0.7236 ± 0.036c |

Note: Data followed by same letter are not significantly different at *p* < .05.

### Table 11. Sodium, potassium and Calcium (mg g⁻¹ dry wt.) contents in *A. sativa* L. cultivar KENT supplied with different salts of potassium (55 DAS).

| Treatments | Sodium | Potassium | Calcium |
|------------|--------|-----------|---------|
|            | Leaf | Root | Stem | Leaf | Root | Stem | Leaf | Root | Stem |
| Control    | 0.977 ± 0.02a | 3.37 ± 1.08a | 8.01 ± 1.56a | 11.86 ± 1.57d | 1.77 ± 0.13a | 9.47 ± 0.48b |
| K₂O 20 kg ha⁻¹ | 0.670 ± 0.17c | 2.40 ± 0.59b | 6.91 ± 1.18d | 14.47 ± 0.75c | 2.46 ± 0.69b | 9.61 ± 0.18a |
| K₂O 40 kg ha⁻¹ | 0.391 ± 0.02f | 1.69 ± 0.35d | 4.51 ± 1.13d | 15.16 ± 0.58a | 2.53 ± 0.54c | 10.81 ± 0.35a |
| K₂O 60 kg ha⁻¹ | 0.419 ± 0.08e | 1.92 ± 0.14c | 3.51 ± 0.35e | 15.86 ± 1.59a | 2.25 ± 0.19c | 11.26 ± 1.48b |
| KNO₃ 20 kg ha⁻¹ | 0.877 ± 0.02b | 1.86 ± 0.08c | 4.60 ± 1.14c | 12.90 ± 0.85a | 2.03 ± 0.23c | 10.87 ± 0.44b |
| KNO₃ 40 kg ha⁻¹ | 0.671 ± 0.06c | 1.73 ± 0.25c | 4.95 ± 0.12b | 13.00 ± 1.53a | 3.29 ± 0.49c | 10.94 ± 0.69a |
| KNO₃ 60 kg ha⁻¹ | 0.647 ± 0.05d | 1.95 ± 0.18b | 3.22 ± 0.98e | 14.51 ± 1.18c | 3.29 ± 0.49c | 11.14 ± 0.59a |

Note: Data followed by same letter are not significantly different at *p* < .05.
potassium has direct effect on the growth and productivity of crop plants (Tomar & Agarwal 2013; Jatav et al. 2014). Enhanced calcium and reduced sodium uptake due to potassium supplementation has been earlier reported in Cicer arietinum L. (Sharma et al. 2006) and wheat (Tomar & Agarwal 2013; Jatav et al. 2014).

Increased nitrate reductase and aminotransferase activity and altered free proline and amino acid contents in potassium treated plants/seedlings in our experiments alongwith improved antioxidant system indicate positive role of potassium in the growth of oat under normal and stress conditions.

Conclusion
Potassium supplementation improved the growth of oat (A. sativa L.) which was evident from the morphological and physiological parameters studied. Increase in contents of phenols and tannins as well as the activities of SOD, CAT and APX in potassium treated plants ensure role of potassium in enhancing and strengthening the antioxidant potential. Increase in synthesis and accumulation of compatible osmolytes like free proline, free sugars and free amino acids and decrease in lipid peroxidation justifies the importance of potassium to crop plants even under stress conditions.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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