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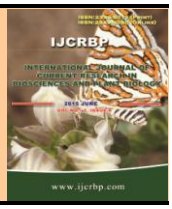

Original Research Article

Kinetin - Polyethyleneglycol Interaction during Seedling Growth and Leaf Disc Senescence in *Cajanus cajan* L.

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Abstract

Seedlings and punched out leaf discs from leaflets of mature pigeonpea plants were used separately to investigate influence of kinetin (Kn) and PEG 6000 separately and also in combination on the growth responses and certain biochemical changes like chlorophylls (chl), protein, RNA DNA and protease activity. Application of Kn was responsible for not only increment in shoot length, seedling diameter and leaf area but also in the amount of chl, protein and nucleic acids in seedlings as well as leaf discs. Different concentrations of PEG 6000, on the other hand, caused varied degree of stress which resulted a sharp decline in various plant metabolites of pigeonpea seedlings and leaf discs; and overall growth due to decrease in cell enlargement and cell division processes. Protein degradation reflected in the larger increment of protease activity. The interaction study clearly showed the effectiveness of Kn to nullify stress effects of PEG 6000 considerably at relatively lower concentration of the latter. Relative changes among the growth parameters were mostly positive except seedling diameter at 6 and 9 day in pigeonpea. The pattern of relative changes in chl a, chl b, total chl, protein, RNA and DNA (mostly) resembled to that of seedling diameter as they depicted negative values at 6 and 9-day. In leaf discs, relative changes were negative at all stages in above parameters except chl b. Chl b and protease activity showed positive changes. Relative changes of RNA:DNA were altogether different in comparison to other parameters.

Keywords

Cajanus cajan
Kinetin-PEG interactions
Leaf-disc senescence
Seedling growth

Introduction

Cytokinins regulate a number of growth and developmental processes in plants such as stimulating cell division, maintaining plant vigour and delaying senescence (Gan and Amasino, 1997; Robson et al., 2004). They are also known to minimize loss of chlorophylls, carotenoids, nucleic acids and proteins (Chen and Kao, 1986; Lamattina et al., 1987; Mukherjee and Kumar, 2007; Seema et al., 2011). On

the other hand, polyethylene glycol (PEG), a polymer produced in a range of molecular weights, can be used to induce plant water deficit in a controlled manner (Lagerwerff et al., 1961). Moreover, PEG of high molecular weight, such as 4000 to 8000 was found to be taken up by plants, the magnitude of uptake was neither identical in different plants nor in different plant organs of the same plant (Lawlor, 1970).

Although various concentrations of PEG has been used to create varied degree of water stress but comparative studies are rare such as to observe the growth pattern and biochemical alterations after PEG and cytokinin (e.g. kinetin) applications. Further, it was thought to be interesting to compare some physiological parameters like chlorophylls, proteins, protease activity, RNA and DNA after either individual application of PEG or Kn or when seedlings and leaf discs are subjected to a combined application. Another aim of this investigation was to compare between absolute and relative changes in all selected parameters as seldom we come across such data in the research papers. By determining the relative increment or decline compared to the previous time point it will also be possible to comment upon the rate of increase or decrease which gives important insight into the progress of physiological responses with time.

Materials and methods

Plant material

Certified pigeonpea seeds (*Cajanus cajan* L. cv. UPAS - 120) obtained from Haryana Agricultural University, Hisar (India) were used either for raising seedlings or growing mature plants. Seedlings and punched out leaf discs from leaflets of mature pigeonpea plants were used separately to carry out experiments with kinetin (Kn) and polyethylene glycol (PEG 6000) as mentioned below:-

(1) Seedling study with Kn:
Seven day old germinated pigeonpea seedlings raised in 45 Petri dishes (9 cm diameter, Borosil-make), divided into 3 equal groups and were treated as follows:
(a) Maintained as untreated control (Kn_0) having 5 ml distilled water in each Petri dish.
(b) Treated set (Kn_1) having 5 ml of 0.005mM Kn in each Petri dish.
(c) Treated set (Kn_2) having 5 ml of 0.05mM Kn is each Petri dish.

(2) Seedling study with PEG 6000:
Seven day old germinated pigeonpea seedlings raised again in 45 Petri dishes as mentioned above, divided into 3 equal groups and were treated as follows:
(a) Maintained as control (S_0) having 5 ml distilled water in each Petri dish.
(b) Treated set (S_1) having 5 ml of 5% PEG (equivalent to -0.70 bars osmotic solution) in each Petri dish.
(c) Treated set (S_2) having 5 ml of 10% PEG (equivalent to -2.0 bars osmotic solution) in each Petri dish.

(3) Seedling study with combined treatment of PEG 6000 and Kn:
Experiment was repeated as with Kn and PEG carried out earlier as mentioned below:
(a) Control set (w_0) having 5 ml distilled water in each Petri dish.
(b) Treated set (w_1) having 5% PEG + 0.05mM Kn; 5 ml of which provided in each Petri dish.
(c) Treated set (w_2) having 10% PEG + 0.05mM Kn; 5 ml of which provided in each Petri dish.
Seven day old seedlings subjected to treatments were considered as 0-day or 'initial' stage in the text and Fig.s.

(4) Leaf discs and PEG-6000:
Leaf discs were punched out from 20-day old tagged leaves of *C. cajan* and maintained in three groups, each group having 20 Petri dishes with floating leaf discs as follows:
(a) Control set (S_0), maintained in distilled water, each Petri dish having 5 ml of it.
(b) Treated set (S_1), maintained in 11.5% PEG (equivalent to -3.0 bars osmotic solution), each Petri dish having 5 ml of it.
(c) Treated set (S_2), maintained in 23.5% PEG (equivalent to -7.5 bars osmotic solution), each Petri dish having 5 ml of it.

(5) Leaf discs, PEG - 6000 and Kn:
Leaf discs were prepared just like previous experiment and were also maintained in three groups, each group having 20 Petri dishes with floating leaf discs as follows:
(a) Control set (S_0), maintained in distilled water, each Petri dish having 5 ml of it.

(b) Treated set (S_x), maintained in 11.5% PEG + 0.05mM Kn, each Petri dish having 5 ml of it.
(c) Treated set (Sy), maintained in 23.5% PEG + 0.05mM Kn, each Petri dish having 5 ml of it.

Leaf area determination

The leaf area was measured by the formula suggested by Stickler et al. (1961) as given below.

$$LA = LW \times LL \times 0.75$$

where, LA = Leaf area, LW = Leaf width, LL = Leaf length, 0.75 = a constant.

Estimation of chlorophylls

Two hundred mg of leaf sample was extracted with 10 ml of chilled 80 per cent acetone (Analytical Reagent, AR grade) and 20 mg of $CaCo_3$ and the filtrate was raised to 10 ml with the same concentration of solvent. The absorbance was recorded at 645 and 663 nm using a spectrophotometer. The pigments were estimated by the formulae and method of Arnon (1949).

Protein estimation and protease activity

Protein was estimated by the method of Bradford (1976) using coomassie brilliant blue G-250 dye. The procedure of protease extraction was a slight modification of that described by Yomo and Varner (1973) and appeared elsewhere (Mukherjee and Kumar, 2007). Protease activity was expressed as nmoles of tyrosine g^{-1} fresh weight h^{-1}.

RNA and DNA estimation

RNA and DNA were determined by a modified method of Ogur and Rosen (1950); the extraction process removed the interfering compounds and involved defatting (Cherry, 1962).

Relative change values

The relative change values were determined for any given parameter using the following formula :-

$$\text{Relative change value (\%)} = \frac{\text{Value of the observed parameter at a specific stage} - \text{Value of the observed parameter at the previous stage}}{\text{Value of the observed parameter at the previous stage}} \times 100$$

Statistical analysis

Each observation and biochemical analysis was based upon three replicates; and for each replicate three aliquots were taken for recording data. The values obtained in each experiment along with the associated standard error values have been given in the Supplemental data: Tables numbered 1-8. The differences in the observed values of parameters with respect to different treatments and with respect to time (days/hours) were checked respectively for their significance using one-way ANOVA, and Tukey's test was performed to find out honestly significant differences. Significance was determined at 5 and 1% probability. The results of Tukey's test have been included in the Supplemental data.

Results

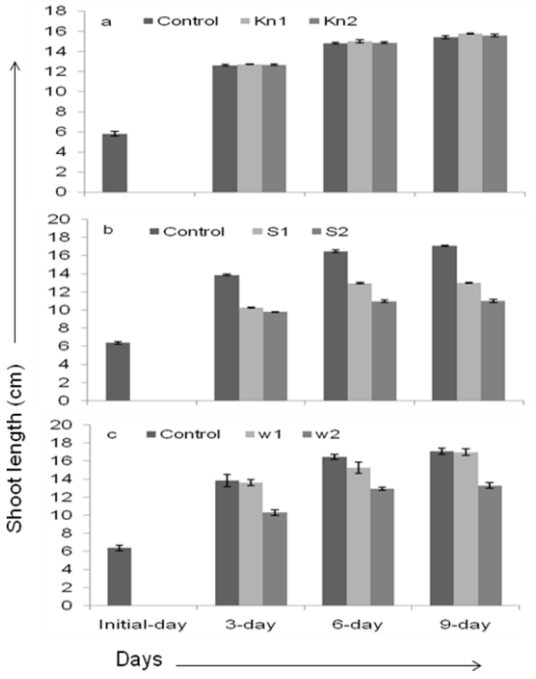
Whole plant experiments

Seedling shoot length: Seedlings were unique in exhibiting very rapid elongation during first three days after starting experiment followed by smaller increase irrespective of treatment (Fig. 1 a-c). Kn concentrations used here did not bring any significant change in the shoot length after three, six and nine days of the treatment compared to the control (Fig. 1a). Under all conditions the growth relative to the previous developmental stage was seen maximum after three days of treatment and this rate declined considerably in successive stages of development (Supp. Fig. 1). No difference was observed in the shoot length between the two Kn concentrations used. PEG treatment reduced the absolute growth and higher concentration induced higher reduction (Fig. 1b). The relative growth under PEG followed the same trend of maximum growth from initial to three-day condition (Supp. Fig. 1). Under the combined treatment of Kn and PEG, Kn was seen to overcome the growth retarding effects of PEG, more efficiently at lower PEG concentration (Fig. 1c).

Seedling diameter: Under all treatments, the seedling diameter was seen to be increasing up to three days, decreased a little at six days and decreased further at nine days (Fig. 2 a-c). The Kn treatment, however, arrested the decline in growth at 9-day stage (Fig. 2a,

Supp. Fig. 2). PEG reduced the growth more prominently at 9-day stage compared to the control (Fig. 2b, Supp. Fig. 2). The trends of absolute and relative values of seedling diameter when PEG was taken alone and when PEG combined with Kn were not very much different (Fig. 2 b-c, Supp. Fig.2).

Fig. 1: *Cajanus cajan* seedlings showing absolute changes in shoot length (in cm) under: (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.



Leaf area: The leaf area was found to be increasing with time, and significant increase was observed at 3-day stage. Under all conditions, the relative growth was found to be maximum at 3-day stage, with sharp decrease at 6-day (Supp. Fig. 3). The growth observed at 6-day and 9-day stages seemed very similar which indicated that the leaf area parameter was not affected much after six days of growth (Fig. 3 a-c). Kn at lower concentration increased both the absolute and relative growth values compared to control (Fig. 3a, Supp. Fig. 3), and higher concentration of PEG decreased the same in isolation as well as in the presence of Kn more prominently at 6-day stage (Fig. 3 b-c, Supp. Fig. 3).

Chlorophyll a: Under all conditions chl a content increased from initial to 3-day stage, then fell below the initial values at 6-day stage and declined further at 9-day stage (Fig. 4 a-c). Kn increased the chl a content at all stages with respect to control, but was found to be more effective at lower concentration (Fig. 4a). Treatment of PEG reduced the chl a content with respect to control and reduction was more pronounced at lower concentration of PEG (Fig. 4c). Relative rates of alterations in chl a were negative at 6 and 9-day stages (Supp. Fig. 4).

Fig. 2: *C. cajan* seedlings showing absolute changes in diameter (in mm) under: (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

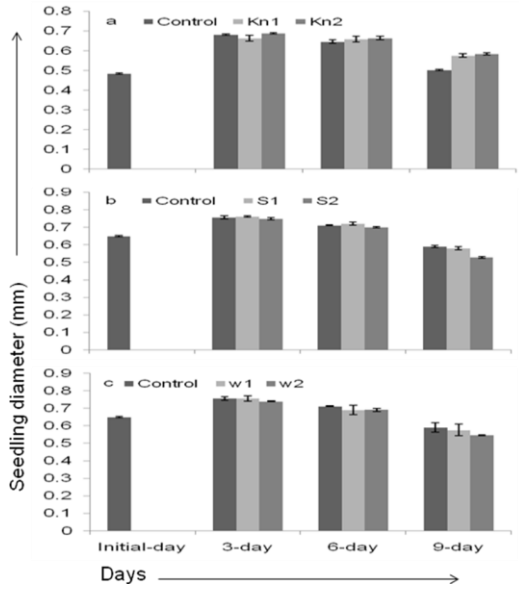
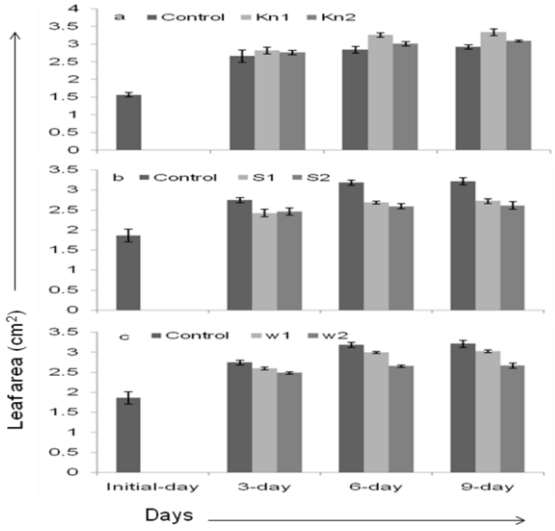


Fig. 3: *C. cajan* seedlings showing absolute changes in leaf area (in cm^2) under: (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.



Chlorophyll b: Chl b content increased from initial to 3-day stage, then fell gradually at 6 and 9-day stages to reach the values closer to initial stage (Fig. 5 a-c). Kn

at low concentration (at 3, 6 and 9-day), and PEG at law concentration (at 6 and 9-day) increased the amount of chl b as compared to control (Fig. 5 a-b). Effect of Kn in presence of PEG was not prominent (Fig. 5c). Relative rates of alterations in chl b were negative at 6 and 9-day stages (Supp. Fig. 5).

Fig. 4: *C. cajan* seedlings showing absolute changes in Chl a of leaves (in mg g^{-1} fr.wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

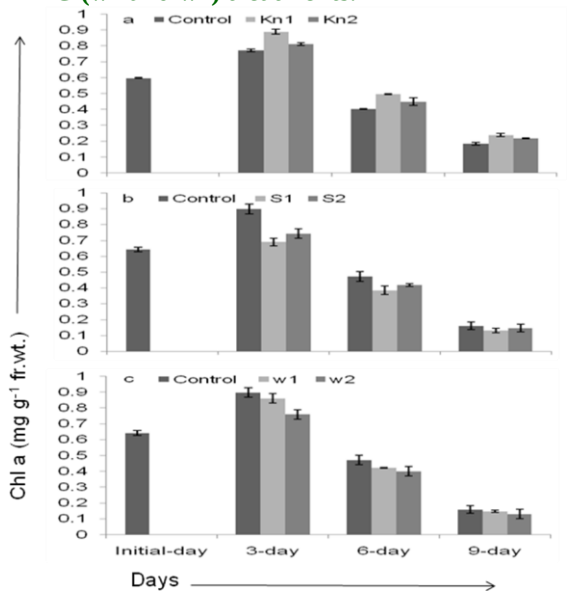
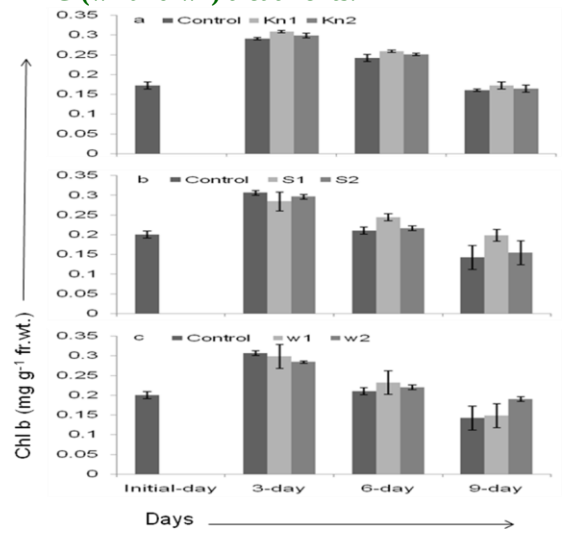


Fig. 5: *C. cajan* seedlings showing absolute changes in Chl b of leaves (in mg g^{-1} fr.wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.



Total chlorophyll: Total chl content profile matched closely with the profile of chl a except at 9-day stage in PEG and, Kn + PEG treatment (Fig. 6 a-c). Relative rate of change in total chl concentration was negative at 6 and 9-day stages (Supp. Fig. 6).

Fig. 6: *C. cajan* seedlings showing absolute changes in Total Chl of leaves (in mg g^{-1} fr.wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

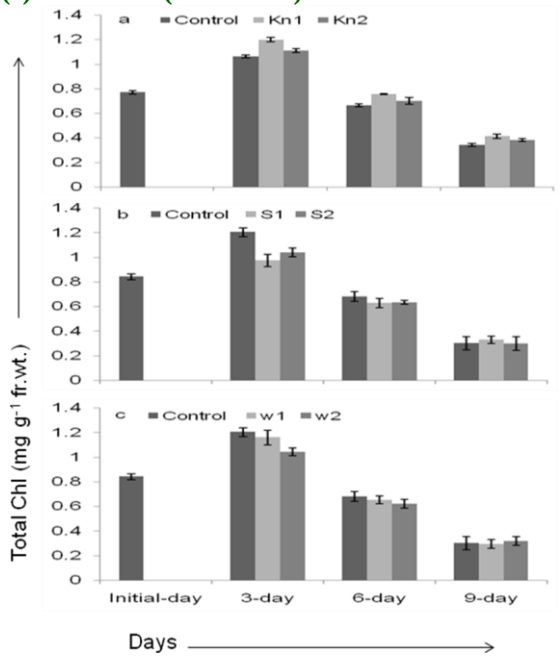


Fig. 7: *C. cajan* seedlings showing absolute changes in Chl a:b ratio of leaves under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

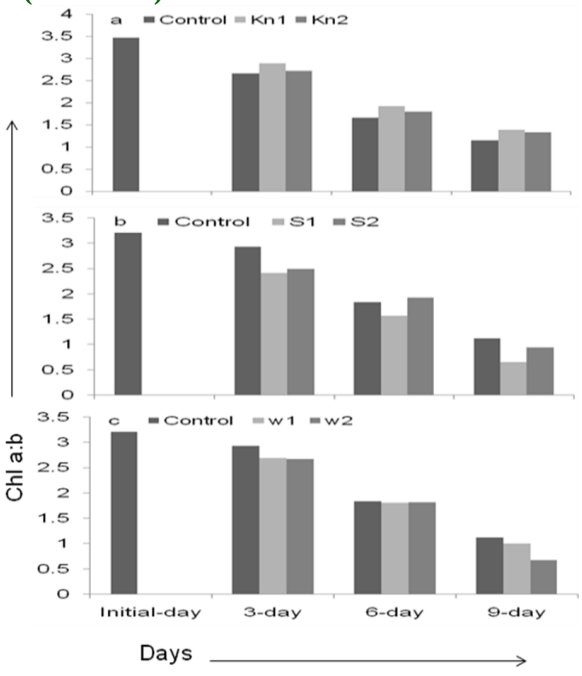


Fig. 8: *C. cajan* seedlings showing absolute changes in protein-content of leaves (in mg /100 mg dry wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

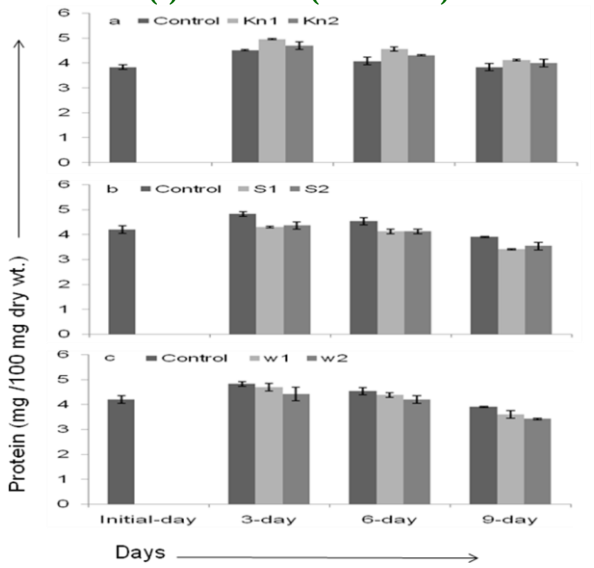
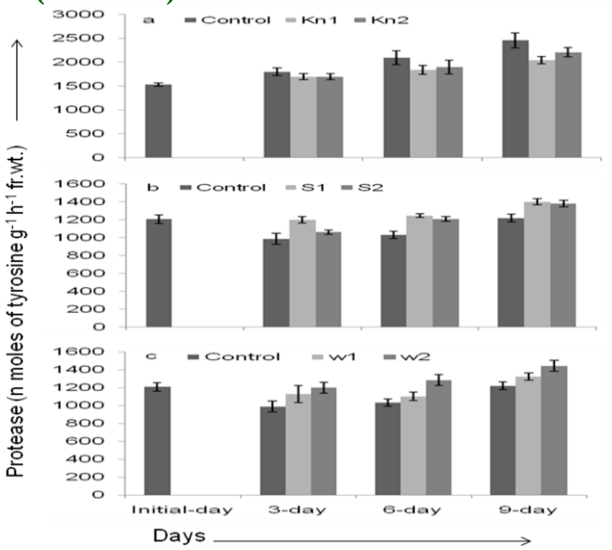
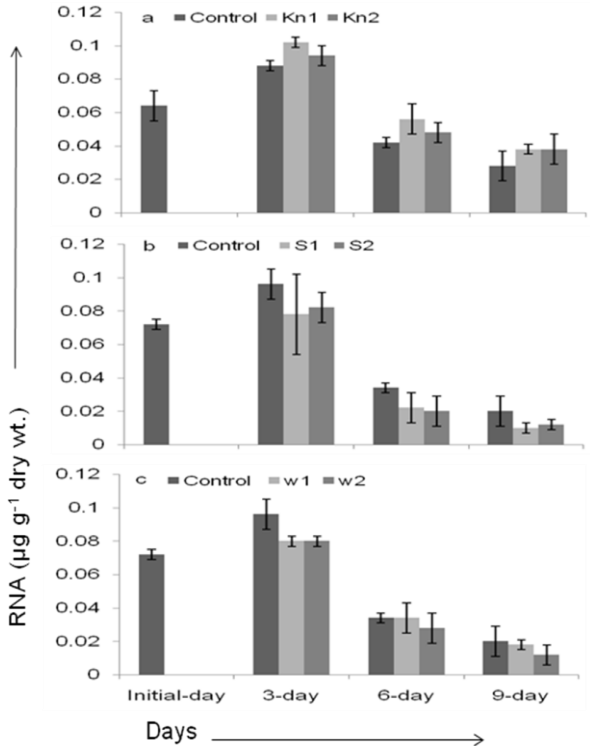


Fig. 9: *C. cajan* seedlings showing absolute changes in protease activity of leaves (measured in nano moles of tyrosine g^{-1} h^{-1} fr.wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.



Chlorophyll a:b ratio: The profile of the ratio also matched closely with that of chl a at individual stages except for 6-day treatment with PEG (Fig. 7 a-c). However, the values were maximum at initial stage, and continuously declined thereafter in 3, 6 and 9-day stages. The relative changes in a:b ratio showed different patterns, though all the values at all stages and treatments remained negative (Supp. Fig. 7). Pigeonpea seedlings having Kn treatment exhibited most negative value at 6-day when relative change in chl a:b ratio was noticed; whereas less negative and least negative values were recorded at 9 and 3-day stages respectively (Supp. Fig. 7). However, in terms of relative change with respect to PEG and Kn + PEG treatments, the negative values kept increasing from 3 to 6 and 6 to 9-day stage (Supp. Fig. 7).

Fig. 10: *C. cajan* seedlings showing absolute changes in RNA-content of leaves (in µg g^{-1} dry wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.



Protein: Kn treatment increased the quantity of protein at all the stages studied with lower concentration showing more pronounced effect (Fig. 8a). The Kn-profile showed a rise at 3-day stage, then successive declines at 6 and 9-day stages with the 9-day values slightly higher than the initial values (Fig. 8a). PEG treatment decreased the protein content with respect to control (Fig. 8b). In combined application of Kn + PEG, higher PEG concentration reduced the protein content more (Fig. 8c). The time course profiles with PEG and Kn + PEG treatments showed a rise at 3-day stage with successive declines at 6 and 9-day with the 9-day values slightly less than the initial values (Fig. 8 b-c). The relative rate of change in protein at all treatments was positive at 3-day stage and negative thereafter. Kn showed a higher relative rate of change at 3-day compared to the control with its lower

concentration being more effective (Supp. Fig. 8). PEG, on the other hand reduced the relative rate of increment at 3-day with respect to control significantly (Supp. Fig. 8), some of this reduction was reversed by additional Kn treatment (Supp. Fig. 8).

Fig. 11: *C. cajan* seedlings showing absolute changes in DNA-content of leaves (in µg g^{-1} dry wt.) under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

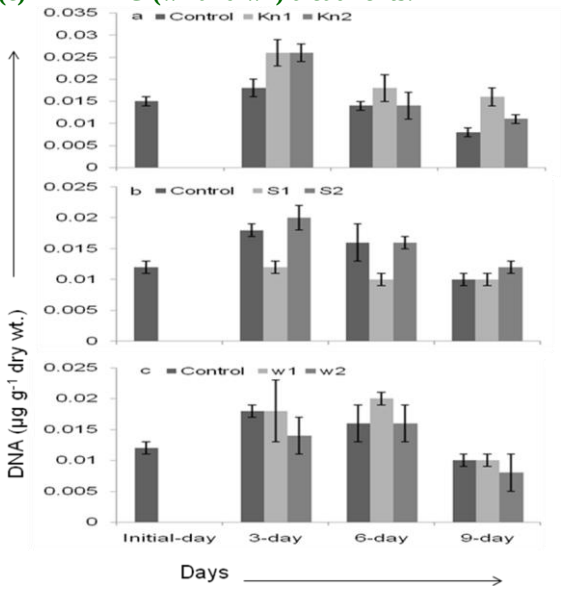


Fig. 12: *C. cajan* seedlings showing absolute changes in RNA:DNA ratio of leaves under; (a) Kn (Kn1 and Kn2) treatments, (b) PEG (S1 and S2) treatments and (c) Kn + PEG (w1 and w2) treatments.

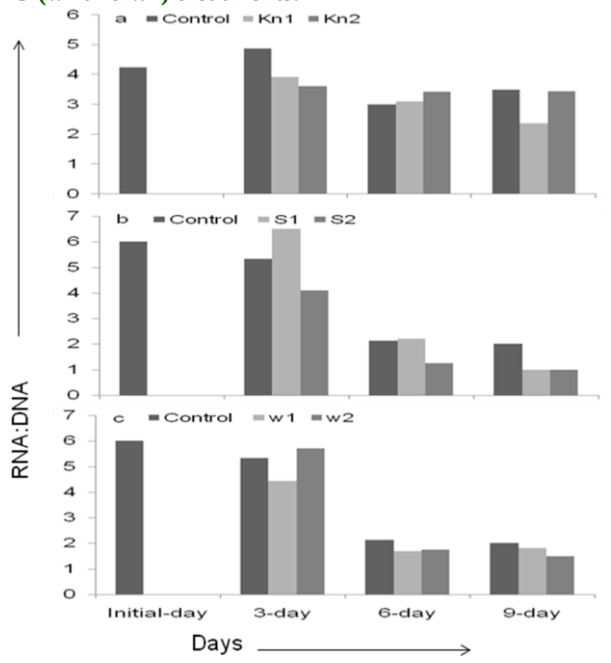


Fig. 13: *C. cajan* leaf discs showing absolute changes in Chl a, measured in mg g^{-1} fr.wt., under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

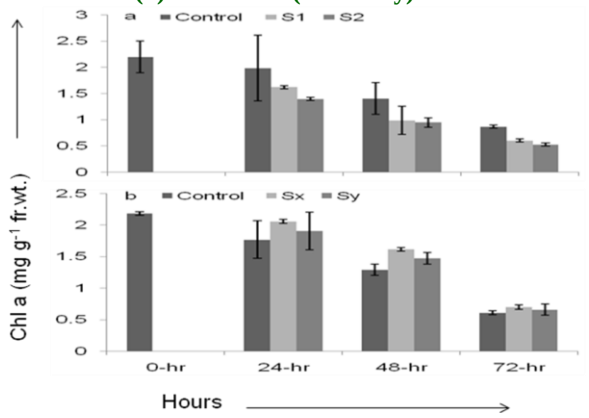


Fig. 14: *C. cajan* leaf discs showing absolute changes in Chl b, measured in mg g^{-1} fr.wt., under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

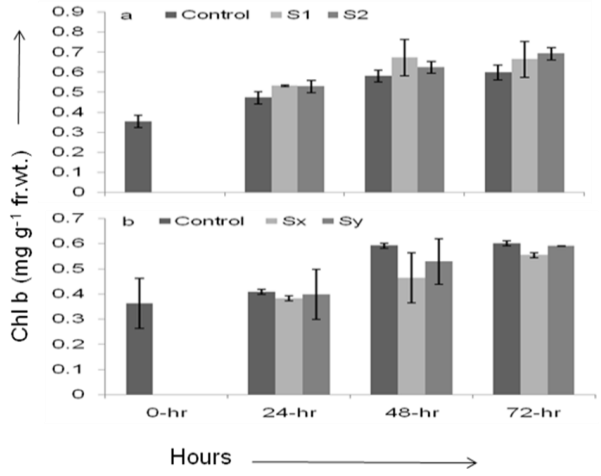


Fig. 15: *C. cajan* leaf discs showing absolute changes in Total Chl, measured in mg g^{-1} fr.wt., under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

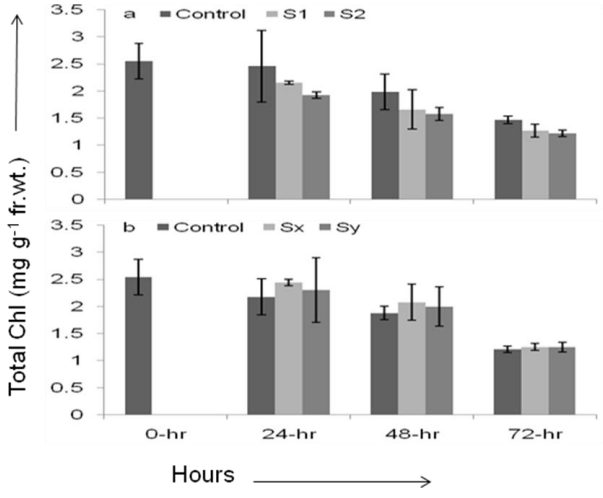


Fig. 16: *C. cajan* leaf discs showing absolute changes in Chl a:b ratio under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

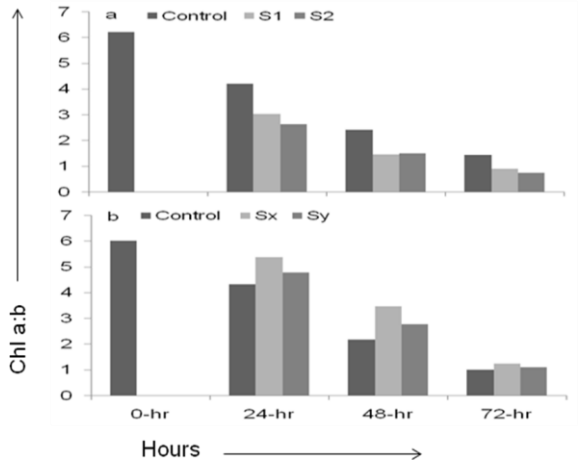


Fig. 17: *C. cajan* leaf discs showing absolute changes in protein-content (measured in mg /100 mg dry wt.) under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

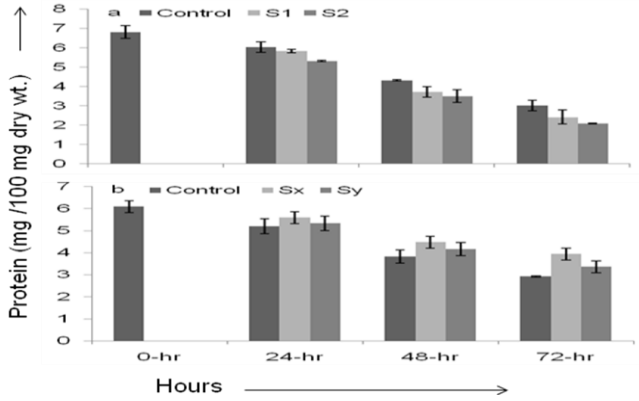


Fig. 18: *C. cajan* leaf discs showing absolute changes in protease activity (measured in nano moles of tyrosine g^{-1} h^{-1} fr.wt.) under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

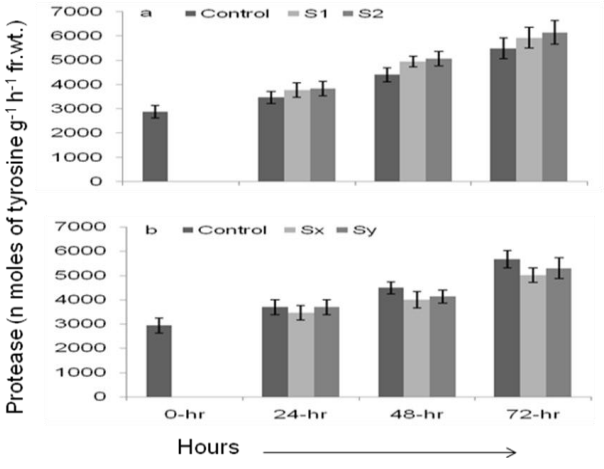


Fig. 19: *C. cajan* leaf discs showing absolute changes in RNA-content (measured in µg g^{-1} dry wt.) under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

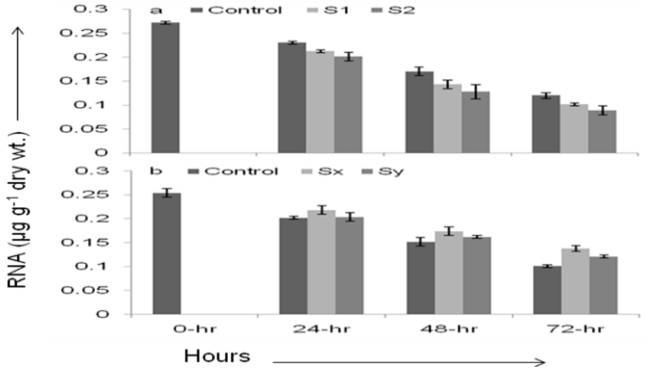


Fig. 20: *C. cajan* leaf discs showing absolute changes in DNA-content (measured in µg g^{-1} dry wt.) under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.

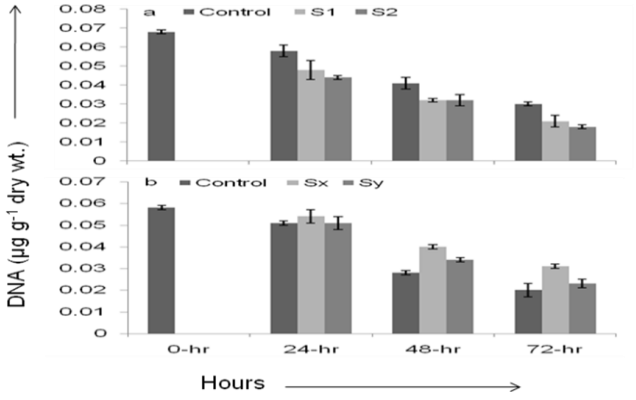
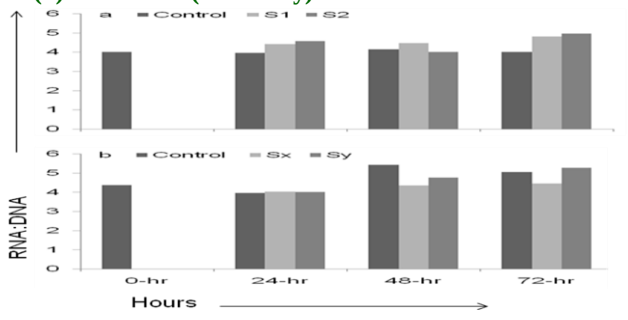


Fig. 21: *C. cajan* leaf discs showing absolute changes in RNA:DNA ratio under; (a) PEG (S1 and S2) treatments and (b) Kn + PEG (Sx and Sy) treatments.



Protease activity: Protease activity was seen to be increasing with time, while Kn treatment reduced this trend to some extent, the lower concentration being more effective (Fig. 9a). However, PEG treatment increased the enzymatic activity with respect to control (Fig. 9b). Kn antagonized the effect of PEG to some degree when low PEG concentration was used

(Fig. 9c). The relative rate of change was positive all along in the Kn treatment, however, in PEG treatment this was negative till 3-day and become positive thereafter (Supp. Fig. 9). Combined application of Kn and PEG resulted a pattern somewhat similar to that of PEG alone (Supp. Fig. 9).

RNA: The amount of RNA increased under all treatments at 3-day stage and decreased thereafter in the later stages (Fig. 10 a-c). Kn increased the RNA with respect to control and its lower concentration seemed more effective (Fig. 10a). PEG decreased the RNA content and it was more effective at lower concentration (Fig. 10b). Kn reduced the effect of PEG at low PEG concentration at 6 and 9-day stage (Fig. 10c). The relative rate of RNA alteration was positive at 3-day stage and negative at 6 and 9-day stage in all treatments, the maximum negative change was recorded at 6-day stage. (Supp. Fig. 10).

DNA: The DNA content increased from initial to 3-day and then gradually declined at 6 and 9-day stages (Fig. 11 a-c). Kn increased the DNA level with lower concentration being more effective (Fig. 11a). Lower concentration of PEG lowered the DNA content while higher concentration interestingly increased it (Fig. 11b). In joint treatment of Kn + PEG, Kn reversed the inhibitory effect of lower PEG concentration and even showed higher amount of DNA than control at 6-day stage (Fig. 11c). Kn with high PEG concentration, however, showed less amount of DNA than control. The relative rates of DNA change were positive at 3-day stage and negative at 6 and 9-day (Supp. Fig. 11). An exception was seen with Kn + PEG treatment, where at 6-day stage, the values were positive (Supp. Fig. 11).

RNA : DNA ratio: The ratio tends to decline with successive stages (Fig. 12 a-c), in PEG treatments the ratio fell sharply at 6-day stage and remained low at 9-day stage (Fig. 12 b-c). The sharp fall in ratio at 6-day stage was also evident from the values of relative change at that stage (Supp. Fig. 12).

Leaf disc experiments

Results of leaf disc experiments of *Cajanus cajan* taking PEG alone and also PEG + Kn have been presented in Figs. 13-21.

Chlorophyll a: The chl a values fell continuously with time, with PEG treatment bringing the values further down (Fig.13a). Kn + PEG treatment increased the amount of chl a with respect to control at every time point, and more so when PEG concentration was low (Fig. 13b). Thus Kn not only antagonized the effect of PEG, but also added some positive effect of its own. The relative rate of change was negative all along, and becoming more negative in successive time points (Supp. Fig. 13).

Chlorophyll b: Chl b values increased with time and PEG treatment increased them further with respect to control (Fig. 14a). In combined application of PEG + Kn, although the values increased with time but treatment values were less than corresponding control values, with higher reduction observed with low PEG concentration (Fig. 14b). The relative rate of change in chl b was positive all along (Fig. 14c), however, in PEG treatment, it was maximum after 24 h, and then declined in successive time points (Supp. Fig. 14). In combined treatment of Kn + PEG, the rate increased from 24 to 48 h condition and then again decreased at 72 h (Supp. Fig. 14).

Total chlorophyll: The total chl content decreased with time and PEG brought about higher reduction than control, while combined treatment of Kn + PEG increased the values with respect to control (Fig. 15 a-b). The relative rate of change in total chl was negative all along and a sharp decline was observed between 48 and 72 h stage under Kn + PEG treatment (Supp. Fig. 15).

Chlorophyll a : b ratio: The ratio decreased with time under both treatments (Fig. 16 a-b) and PEG brought down the values further with respect to control (Fig. 16a), while combined treatment of PEG + Kn, the ratio was higher in treatments with respect to control, the effect more pronounced at lower PEG concentration (Fig. 16b). Thus Kn seemed to antagonize the effect of PEG. The relative rates of change in a:b ratio were negative all along (Supp. Fig. 16).

Protein: The amount of protein decreased with time and PEG brought it further down with respect to control (Fig. 17a). In joint treatment of PEG + Kn, the declining trend was similar; however, the treatment values were higher than the corresponding control values (Fig. 17b). Thus, Kn seemed to antagonize the

effect of PEG and more so when latter's concentration was low.

The values of relative rate of change were negative under all conditions (Supp. Fig. 17). In PEG treatment, they became more negative as time progressed, while in combined treatment, the negative rate of change increased from 1 to 2-day stage and then decreased a little at 3-day compared to 2-day stage (Supp. Fig. 17).

Protease activity: The protease activity increased with time under both experiments (Fig. 18 a-b); PEG in a dose-dependent manner increased it further with respect to control (Fig. 18a) while in joint treatment, Kn brought down the values with respect to the control values (Fig. 18b). The relative rates of change were positive all along but trends varied among the two experiments (Supp. Fig. 18).

RNA: The RNA content decreased with time (Fig. 19 a-b), with PEG in a dose-dependent manner, decreased it further with respect to control (Fig. 19a). Under combined application, the treatment values were higher than the corresponding control values. The relative rates of change were negative throughout, and in PEG treatment, maximum negative value was noticed at 2-day, while in combined treatment, it was seen at 3-day stage (Supp. Fig. 19).

DNA: The DNA concentration also decreased with time; with PEG decreasing it further down with respect to control (Fig. 20a). In combined application of PEG + Kn also, the DNA content went down with time, however, treatment values were higher than the corresponding untreated control values (Fig. 20b). At lower concentration of PEG, Kn was more efficient in its antagonistic effect. The values of relative rate of change in DNA were negative under all conditions, but trends showed variations among the two experiments (Supp. Fig. 20).

RNA : DNA ratio: PEG increased the ratio of RNA-DNA with respect to control at all time points (Fig. 21a) while in combined treatment, reduction was seen at 48-hr and 72-h stages with low PEG concentration, and reduction with respect to control was also observed at 48-h stage for high PEG concentration (Fig. 21b). The trends of relative rates of change varied considerably among the two experiments (Supp. Fig. 21). Generally, a positive rate was observed in PEG treatment, while in the combined application, negative rate of change was observed at 24-h stage, which reversed to high positive rate at 48-h stage (Supp. Fig. 21).

Discussion

A comparison between results obtained in relation to shoot length after PEG 6000 and Kn treatments indicated that the former was highly effective in inducing stress and reducing the length; the higher concentration causing more inhibition than the lower one (Fig. 1 a-c). PEG seems to adjust not only osmotic potential but also responsible for degradation of protein, RNA and DNA (Fig. 8 a-c, 10 a-c, 11 a-c) as revealed in this study. Stress is responsible for inhibition of cell elongation / cell division or both. Stress is also responsible for decrease in total chl content (Fig. 6 b-c). Reduction in seedling growth as a result of water stress has been reported earlier also (Aspinall et al., 1967; Seehy et al., 1975; Basra et al., 1988). The decrease in seedling diameter and leaf area would most likely result due to diminished cell enlargement or inhibition of cell division or both (Hsiao, 1973). The relative changes in shoot length, seedling diameter and leaf area (Supp. Fig. 1, 2, 3). were found to be positive during first three days of treatment, while values fell down rapidly and negative rates were also observed in case of seedling diameter.

Growth processes in this study, however, stimulated in presence of both the concentrations of Kn. But marked rise could not be seen in shoot length. The increase in leaf area and seedling diameter may be due to enhancement in cell division and increase in protein synthesis or due to inhibition of growth retarding hormones (Miller, 1956; Kulaeva and Tsybulya, 1974; Goring et al., 1984). Reports are available showing inhibitory effect (Saxena and Maheshwari, 1979), no effect (Stapfer and Heuser, 1986) and stimulatory effect (Richards and Wilkinson, 1984; Miller and Eldridge, 1986) of Kn on shoot growth. Increment in the amount of protein, RNA and DNA has been noticed in the present investigation.

Interaction of PEG and Kn revealed effectiveness of 0.005 mM Kn in minimizing stress phenomenon of PEG as witnessed in length and diameter of seedlings and leaf area at lower concentration only. This trend also continued in the amount of chlorophylls, protein, RNA and DNA where Kn was found to bring down the stress effect of PEG (5%) at lower concentration.

Table 1. *C. cajan* seedlings showing changes (Mean ± S.E.) in various growth parameters after Kn treatments.

Stages after treatment	Shoot length (cm)			Seedling diameter (mm)			Leaf area (cm^2)		
	Kn_0	Kn_1	Kn_2	Kn_0	Kn_1	Kn_2	Kn_0	Kn_1	Kn_2
0-Day	5.83 ±0.08	-	-	0.484 ±0.001	-	-	1.564 ±0.021	-	-
3-Day	12.64 ±0.03	12.72 ±0.01	12.68 ±0.03	0.682 ±0.001	0.664 ±0.005	0.689 ±0.001	2.66 ±0.058	2.82 ±0.032	2.76 ±0.021
6-Day	14.84 ±0.03	15.02 ±0.05	14.88 ±0.03	0.646 ±0.003	0.659 ±0.005	0.664 ±0.003	2.84 ±0.032	3.26 ±0.021	3.01 ±0.021
9-Day	15.42 ±0.05	15.78 ±0.01	15.60 ±0.05	0.502 ±0.001	0.576 ±0.003	0.584 ±0.002	2.92 ±0.021	3.34 ±0.032	3.09 ±0.010

Kn_0 = Control; Kn_1 = 0.005 mM kinetin; Kn_2 = 0.05 mM kinetin.

Tukey's HSD Test

Day wise

1) Column 1 : Shoot length (Kn_0). All comparisons significant ($p<0.01$).
2) Column 2 : Shoot length (Kn_1). All comparisons significant ($p<0.01$).
3) Column 3 : Shoot length (Kn_2). All comparisons significant ($p<0.01$).
4) Column 4 : Seedling diameter (Kn_0). All comparisons significant ($p<0.01$).
5) Column 5 : Seedling diameter (Kn_1). All but one comparisons significant ($p<0.01$); 3-day versus 6-day – non significant (ns).
6) Column 6 : Seedling diameter (Kn_2). All comparisons significant ($p<0.01$).
7) Column 7 : Leaf area (Kn_0). All but one comparisons significant ($p<0.01$); 6-day versus 9-day (ns).
8) Column 8 : Leaf area (Kn_1). All but one comparisons significant ($p<0.01$); 6-day versus 9-day (ns).
9) Column 9 : Leaf area (Kn_2). All comparisons significant ($p<0.01$); but 6-day versus 9-day significant at $p<0.05$.

Treatment wise

1) Shoot length (3-day). All comparisons ns.
2) Shoot length (6-day) Kn_0 vs. Kn_1 significant ($p<0.01$)
   Kn_1 vs. Kn_2 significant ($p<0.05$)
   Kn_0 vs. Kn_2 ns
3) Shoot length (9-day) Kn_0 vs. Kn_1 significant ($p<0.01$)
   Kn_0 vs. Kn_2 significant ($p<0.05$)
   Kn_1 vs. Kn_2 significant ($p<0.05$)
4) Seeding diameter (3-day). All but one comparisons significant at $p<0.01$; Kn_0 versus Kn_2 (ns).
5) Seeding diameter (6-day). All comparisons ns.
6) Seeding diameter (9-day). All but one comparisons significant at $p<0.01$; Kn_1 versus Kn_2 (ns.)
7) Leaf area (3-day). Only one comparison significant at ($p<0.05$). Kno versus Kn_1 significant. Rest comparisons ns.
8) Leaf area (6-day). All comparisons significant at ($p<0.01$).
9) Leaf area (9-day). All comparisons significant at ($p<0.01$).

Table 2. *C. cajan* seedlings showing changes in chlorophyll contents (mg g^{-1} fr. wt. ± S.E.), protein (mg/100 mg dry wt. ± S.E.), protease activity (n moles of tyrosine g^{-1} h^{-1} fr. wt. ± S.E.), RNA and DNA (µg g^{-1} dry wt. ± S.E.) in leaves after Kn treatments.

Leaf constituents	0-day	3-day			6-day			9-day		
	Kn_0	Kn_0	Kn_1	Kn_2	Kn_0	Kn_1	Kn_2	Kn_0	Kn_1	Kn_2
Chl-a	0.598 ±0.001	0.772 ±0.003	0.890 ±0.005	0.812 ±0.003	0.402 ±0.001	0.498 ±0.00	0.450 ±0.008	0.184 ±0.003	0.240 ±0.003	0.218 ±0.001
Chl-b	0.172 ±0.003	0.290 ±0.001	0.308 ±0.001	0.298 ±0.002	0.242 ±0.003	0.259 ±001	0.251 ±0.001	0.160 ±0.001	0.172 ±0.003	0.164 ±0.003
Total Chl	0.770 ±0.004	1.062 ±0.004	1.198 ±0.006	1.110 ±0.005	0.644 ±0.004	0.757 ±0.001	0.701 ±0.009	0.344 ±0.004	0.412 ±0.006	0.382 ±0.004
Chl a:b	3.47	2.66	2.89	2.72	1.66	1.92	1.79	1.15	1.39	1.33
Protein	3.84 ±0.03	4.52 ±0.01	4.96 ±0.01	4.70 ±0.05	4.08 ±0.05	4.56 ±0.03	4.32 ±0.009	3.84 ±0.05	4.12 ±0.01	4.00 ±0.05
Protease activity	1532 ±10.8	1802 ±26.2	1698 ±20.8	1698 ±20.8	2098 ±48.0	1840 ±32.4	1898 ±48.0	2460 ±52.4	2042 ±26.2	2208 ±32.4
RNA	0.064 ±0.003	0.088 ±0.001	0.102 ±0.001	0.094 ±0.002	0.042 ±0.001	0.056 ±0.003	0.048 ±0.002	0.028 ±0.003	0.038 ±0.001	0.038 ±0.003
DNA	0.015 ±0.001	0.018 ±0.002	0.026 ±0.003	0.026 ±0.002	0.014 ±0.001	0.018 ±0.003	0.014 ±0.003	0.008 ±0.001	0.016 ±0.002	0.011 ±0.001
RNA : DNA	4.24	4.88	3.92	3.61	3.00	3.11	3.42	3.50	2.37	3.45

Kn_0 = Control; Kn_1 = 0.005 mM Kn; Kn_2 = 0.05 mM Kn.

Day wise

1) Chl a (Kn_o). All comparisons significant (p<0.01)
2) Chl a (Kn_1). All comparisons significant (p<0.01)
3) Chl a (Kn_2). All comparisons significant (p<0.01)
4) Chl b (Kn_0). All comparisons significant (p<0.01)
5) Chl b (Kn_1). All but one comparisons significant (p<0.01); initial day versus 9-day (ns).
6) Chl b (Kn_2). All but one comparisons significant (p<0.01); initial day versus 9-day (ns).
7) Protein (Kn_0). All but one comparisons significant (p<0.01); Initial day versus 9-day (ns).
8) Protein (Kn_1). All comparisons significant (P<.01).
9) Protein (Kn_2). All comparisons significant. Initial versus 9-day significant at p<0.01, rest all significant at p<0.01.
10) Protease activity (Kn_0). All comparisons significant (p<0.01).
11) Protease activity (Kn_1). All comparisons significant (p<0.01).
12) Protease activity (Kn_2). All comparisons significant (p<0.01).
13) RNA (Kn_0). All comparisons significant (p<0.01).
14) RNA (Kn_1). All but one comparisons significant (p<0.01); Initial day versus 6-day (ns).
15) RNA (Kn_2). All comparisons significant (p<0.01).
16) DNA (Kn_0). All comparisons significant (p<0.01).
17) DNA (Kn_1). All comparisons significant (p<0.01).
18) DNA (Kn_2). All comparisons significant (p<0.01).

Treatment wise

1) Chl a (3-day). All comparisons significant (p<0.01).
2) Chl a (6-day). All comparisons significant (p<0.01).
3) Chl a (9-day). All comparisons significant (p<0.01).
4) Chl b (3-day). All but one comparisons significant (P<.01).
5) Chl b (6-day). Only one comparison significant (p<0.01); Kn_0 versus Kn_1 significant. All other comparisons ns.
6) Chl b (9-day). All comparisons significant (p<0.01).
7) Protein (3-day). All comparisons significant (p<0.01).
8) Protein (6-day). All comparisons significant (p<0.01).
9) Protein (9-day). Kn_0 vs Kn_1 significant (p<0.01).
   Kn_0 vs Kn_2 significant (p<0.01).
   Kn_1 vs Kn_2 ns
10) Protease (3-day). All but one comparisons significant (p<0.01); Kn_1 versus Kn_2 (ns).
11) Protease (6-day). All but one comparisons significant (p<0.01); Kn_1 versus Kn_2 (ns).
12) Protease (9-day). All comparisons significant
   Kn_0 vs Kn_1 significant (p<0.01)
   Kn_0 vs Kn_2 significant (p<0.01)
   Kn_1 vs Kn_2 significant (p<0.01)
13) RNA (3-day). All comparisons significant (p<0.01).
14) RNA (6-day). All comparisons significant (p<0.01).
15) RNA (9-day). All comparisons significant (p<0.01).
16) DNA (3-day). All comparisons significant (p<0.01).
17) DNA (6-day). All comparisons (ns).
18) DNA (9-day). All comparisons significant (p<0.01).

Table 3. *C. cajan* seedlings showing changes (Mean ± S.E.) in various growth parameters under varying degrees of water stress induced by PEG.

Stages after stress induction	Shoot length (cm)			Seedling diameter (mm)			Leaf area (cm^2)		
	S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2
0-Day	6.40 ±0.05	-	-	0.650 ±0.001	-	-	1.860 ±0.052	-	-
3-Day	13.87 ±0.03	10.30 ±0.02	9.82 ±0.01	0.756 ±0.003	0.762 ±0.001	0.750 ±0.002	2.740 ±0.021	2.420 ±0.032	2.460 ±0.030
6-Day	16.48 ±0.05	12.98 ±0.03	10.98 ±0.05	0.712 ±0.001	0.722 ±0.003	0.701 ±0.001	3.181 ±0.021	2.680 ±0.012	2.590 ±0.021
9-Day	17.10 ±0.02	13.02 ±0.02	11.01 ±0.05	0.591 0.002	0.580 ±0.003	0.528 ±0.002	3.210 ±0.030	2.720 ±0.020	2.610 ±0.032

S_0 = Control; S_1 = 5% PEG; S_2 = 10% PEG.

Day wise

1) Shoot length (S_0). All comparisons significant (p<0.01).
2) Shoot length (S_1). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
3) Shoot length (S_2). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
4) Seedling diameter (S_0). All comparisons significant (p<0.01).
5) Seedling diameter (S_1). All comparisons significant (p<0.01).
6) Seedling diameter (S_2). All comparisons significant (p<0.01).
7) Leaf area (S_0). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
8) Leaf area (S_1). All but one comparisons significant (P<.01); 6-day versus 9-day (ns).
9) Leaf area (S_2).

Initial day vs. 3-day significant (P<.01); 3-day vs 9-day significant (p<0.05)

Initial day vs. 6-day significant (p<0.01); 3-day vs 6-day ns

Initial day vs. 9-day significant (p<0.01); 6-day vs 9-day ns

Treatment wise

1) Shoot length (3-day). All comparisons significant (p<0.01).
2) Shoot length (6-day). All comparisons significant (p<0.01).
3) Shoot length (9-day). All comparisons significant (p<0.01).
4) Seedling diameter (3-day). All comparisons significant (p<0.01).
5) Seedling diameter (6-day). All comparisons significant (p<0.01).
6) Seedling diameter (9-day). All comparisons significant (p<0.01).
7) Leaf area (3-day). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
8) Leaf area (6-day). All comparisons significant (p<0.01).
9) Leaf area (9-day). All comparisons significant.

S_0 vs S_1 significant (p<0.01)

S_0 vs S_2 significant (p<0.01)

S_1 vs S_2 significant (p<0.05)

Table 4. *C. cajan* seedlings showing changes in chlorophyll contents (mg g^{-1} fr. wt. ± S.E.), protein (mg/100 mg dry wt. ± S.E.), protease activity (n moles of tyrosine g^{-1} hr^{-1} fr. wt. ± S.E.), RNA and DNA (µg g^{-1} dry wt. ± S.E.) in leaves under varying degrees of water stress induced by PEG.

Leaf constituents	0-day	3-day			6-day			9-day		
	S_0	S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2
Chl-a	0.642 ±0.005	0.898 ±0.008	0.690 ±0.008	0.742 ±0.010	0.472 ±0.010	0.384 ±0.003	0.418 ±0.003	0.160 ±0.008	0.131 ±0.005	0.146 ±0.008
Chl-b	0.200 ±0.003	0.306 ±0.002	0.284 ±0.008	0.296 ±0.002	0.210 ±0.003	0.244 ±003	0.216 ±0.002	0.142 ±0.010	0.198 ±0.005	0.154 ±0.010
Total Chl	0.842 ±0.008	1.204 ±0.012	0.974 ±0.016	1.038 ±0.012	0.682 ±0.013	0.628 ±0.012	0.634 ±0.005	0.302 ±0.018	0.329 ±0.010	0.300 ±0.018
Chl a:b	3.21	2.93	2.42	2.50	1.84	1.57	1.93	1.12	0.66	0.94
Protein	4.20 ±0.05	4.82 ±0.03	4.30 ±0.01	4.36 ±0.05	4.53 ±0.05	4.12 ±0.03	4.12 ±0.03	3.90 ±0.01	3.41 ±0.01	3.54 ±0.05
Protease activity	1208 ±16.02	988 ±20.4	1198 ±12.2	1062 ±8.12	1032 ±13.6	1246 ±6.86	1208 ±9.04	1220 ±14.2	1402 ±10.8	1380 ±12.2
RNA	0.072 ±0.001	0.096 ±0.003	0.078 ±0.008	0.082 ±0.003	0.034 ±0.001	0.022 ±0.003	0.020 ±0.003	0.020 ±0.003	0.010 ±0.001	0.012 ±0.001
DNA	0.012 ±0.001	0.018 ±0.001	0.012 ±0.001	0.020 ±0.002	0.016 ±0.003	0.010 ±0.001	0.016 ±0.001	0.010 ±0.001	0.010 ±0.001	0.012 ±0.001
RNA : DNA	6.00	5.33	6.50	4.10	2.12	2.2	1.25	2.0	1.0	1.0

S_0 = Control; S_1 = 5% PEG; S_2 = 10% PEG.

Day wise

1) Chl a (S_0). All comparisons significant (p<0.01).
2) Chl a (S_1). All comparisons significant (p<0.01).
3) Chl a (S_2). All comparisons significant (p<0.01).
4) Chl b (S_0). All but one comparisons significant (p<0.01); initial day versus 6-day (ns).
5) Chl b (S_1). All but one comparisons significant (p<0.01); initial day versus 9-day (ns).
6) Chl b (S_2). All but one comparisons significant (p<0.01); Initial vs 6-day (ns).
7) Protein (S_0). All comparisons significant (P<.01).
8) Protein (S_1). All but two comparisons significant (p<0.01); Initial day versus 3-day (ns); Initial day versus 6-day (ns).
9) Protein (S_2). All but two comparisons significant (p<0.01); Initial day versus 3-day (ns); Initial day versus 6-day (ns).
10) Protease Activity (S_0). All but two comparisons significant (P<.01); Initial day versus 9-day (ns); 3-day versus 6-day (ns).
11) Protease activity (S_1). Initial day vs 9-day significant (p<0.01).
    3-day vs 6-day significant (p<0.05).
    3-day vs 9-day significant (p<0.01).
    6-day vs 9-day significant (p<0.01).
    Initial day vs 3-day ns
    Initial day vs 6-day ns
12) Protease activity (S_2). All but one comparisons significant (p<0.01); Initial day versus 6-day (ns).
13) RNA (S_0). All comparisons significant (p<0.01).
14) RNA (S_1). All but two comparisons significant (p<0.01); Initial day versus 3-day (ns); 6-day versus 9-day (ns).
15) RNA (S_2). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
16) DNA (S_0). All comparisons significant (p<0.01).
17) DNA (S_1). All comparisons (ns).
18) DNA (S_2). All comparisons significant (p<0.01).

Treatment wise

1) Chl a (3-day). All comparisons significant (p<0.01).
2) Chl a (6-day). All but one comparisons significant (p<0.01) S_1 versus S_2 (ns).
3) Chl a (9-day). All comparisons ns.
4) Chl b (3-day). Only one comparison significant (p<0.01); S_0 versus S_1 significant.
5) Chl b (6-day). All but one comparisons significant (p<0.01); S_0 versus S_2 (ns).
6) Chl b (9-day). S_0 versus S_1 significant (p<0.01).
    S_1 versus S_2 significant (p<0.05).
    S_0 versus S_2 ns.
7) Protein (3-day). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
8) Protein (6-day). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
9) Protein (9-day). All comparisons significant.
    S_0 versus S_1 significant (p<0.01).
    S_0 versus S_2 significant (p<0.01).
    S_1 versus S_2 significant (p<0.05).
10) Protease activity (3-day). All comparisons significant (p<0.01).
11) Protease activity (6-day). All comparisons significant.
    S_0 versus S_1 significant (p<0.01).
    S_0 versus S_2 significant (p<0.01).
    S_1 versus S_2 significant (p<0.05).
12) Protease activity (9-day). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
13) RNA (3-day). All comparisons ns.
14) RNA (6-day). All but one comparisons significant (p<0.05); S_1 versus S_2 (ns).
15) RNA (9-day). All comparisons significant (p<0.01).
16) DNA (3-day). All comparisons significant (p<0.01).
17) DNA (6-day). All comparisons (ns).
18) DNA (9-day). All comparisons significant (p<0.01).

Table 5. *C. cajan* seedlings showing changes (value ± S.E.) in various growth parameters under combined treatment of PEG and Kn.

Stages after treatment	Shoot length (cm)			Seedling diameter (mm)			Leaf area (cm^2)		
	w_0	w_1	w_2	w_0	w_1	w_2	w_0	w_1	w_2
0-Day	6.40 ±0.10	-	-	0.650 ±0.001	-	-	1.860 ±0.052	-	-
3-Day	13.87 ±0.23	13.64 ±0.12	10.32 ±0.10	0.756 ±0.003	0.756 ±0.005	0.740 ±0.001	2.740 ±0.021	2.598 ±0.012	2.484 ±0.010
6-Day	16.48 ±0.10	15.30 ±0.21	12.96 ±0.05	0.712 ±0.001	0.690 ±0.009	0.692 ±0.003	3.180 ±0.021	2.990 ±0.009	2.650 ±0.010
9-Day	17.10 ±0.12	17.01 ±0.12	13.32 ±0.10	0.591 ±0.009	0.576 ±0.011	0.546 ±0.001	3.210 ±0.030	3.020 ±0.012	2.664 ±0.021

w_0 = Control; w_1 = 5% PEG + 0.005mM Kn; w_2 = 10% PEG + 0.005 mM Kn

Day wise

1) Shoot length (w_0). All comparisons significant (p<0.01).
2) Shoot length (w_1). All comparisons significant (p<0.01).
3) Shoot length (w_2). All comparisons significant (p<0.01).
4) Seedling diameter (w_0). All comparisons significant (p<0.01).
5) Seedling diameter (w_1). All but one comparisons significant (p<0.01). Initial day versus 6-day (ns).
6) Seedling diameter (w_2). All comparisons significant (p<0.01).
7) Leaf area (w_0). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
8) Leaf area (w_1). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).
9) Leaf area (w_2). All but one comparisons significant (p<0.01); 6-day versus 9-day (ns).

Treatment wise

1) Shoot length (3-day). All but one comparisons significant (p<0.01). w_0 versus w_1 (ns).
2) Shoot length (6-day). All comparisons significant (p<0.01).
3) Shoot length (9-day). All but one comparisons significant (p<0.01); w_0 versus w_1 (ns).
4) Seedling diameter (3-day). All but one comparisons significant (p<0.01); w_0 versus w_1 (ns).
5) Seedling diameter (6-day). All but one comparisons significant (p<0.01); w_1 versus w_2 (ns).
6) Seedling diameter (9-day). Only one comparison significant (p<0.01); w_0 versus w_2 significant.
7) Leaf area (3-day). All comparisons significant (p<0.01).
8) Leaf area (6-day). All comparisons significant (p<0.01).
9) Leaf area (9-day). All comparisons significant (p<0.01).

Table 6. *C. cajan* seedlings showing changes in chlorophyll contents (mg g^{-1} fr. wt. ± S.E.), protein (mg/100 mg dry wt. ± S.E.), protease activity (n moles of tyrosine g^{-1} hr^{-1} fr. wt. ± S.E.), RNA and DNA (µg g^{-1} dry wt. ± S.E.) in leaves under combined treatment of PEG and Kn.

Leaf constituents	0-day	3-day			6-day			9-day		
	w_0	w_0	w_1	w_2	w_0	w_1	w_2	w_0	w_1	w_2
Chl-a	0.642 ±0.005	0.898 ±0.010	0.862 ±0.010	0.760 ±0.010	0.472 ±0.010	0.422 ±0.000	0.401 ±0.010	0.160 ±0.008	0.148 ±0.002	0.130 ±0.010
Chl-b	0.200 ±0.003	0.306 ±0.002	0.298 ±0.010	0.284 ±0.001	0.210 ±0.003	0.232 ±010	0.220 ±0.002	0.142 ±0.010	0.148 ±0.010	0.190 ±0.002
Total Chl	0.842 ±0.008	1.204 ±0.012	1.160 ±0.020	1.044 ±0.011	0.682 ±0.013	0.654 ±0.010	0.621 ±0.012	0.302 ±0.018	0.296 ±0.012	0.320 ±0.012
Chl a:b	3.21	2.93	2.69	2.67	1.84	1.81	1.82	1.12	1.00	0.68
Protein	4.20 ±0.05	4.82 ±0.03	4.69 ±0.05	4.42 ±0.09	4.53 ±0.05	4.38 ±0.03	4.20 ±0.05	3.90 ±0.01	3.60 ±0.05	3.42 ±0.01
Protease activity	1208 ±16.02	988 ±20.4	1128 ±32.4	1198 ±20.4	1032 ±13.6	1102 ±16.0	1284 ±20.4	1220 ±14.2	1324 ±13.6	1442 ±20.4
RNA	0.072 ±0.001	0.096 ±0.003	0.080 ±0.001	0.080 ±0.001	0.034 ±0.001	0.034 ±0.003	0.028 ±0.003	0.020 ±0.003	0.018 ±0.001	0.012 ±0.002
DNA	0.012 ±0.001	0.018 ±0.001	0.018 ±0.005	0.014 ±0.003	0.016 ±0.003	0.020 ±0.001	0.016 ±0.003	0.010 ±0.001	0.010 ±0.001	0.008 ±0.003
RNA : DNA	6.00	5.33	4.44	5.71	2.12	1.70	1.75	2.00	1.80	1.50

w_0 = Control; w_1 = 5% PEG + 0.005mM Kn; w_2 = 10% PEG + 0.005 mM Kn.

Day wise

1) Chl a (w_0). All comparisons significant (p<0.01).
2) Chl a (w_1). All comparisons significant (p<0.01).
3) Chl a (w_2). All comparisons significant (p<0.01).
4) Chl b (w_0). All but one comparisons significant (p<0.01); Initial day versus 6-day (ns).
5) Chl b (w_1). All but one comparisons significant (p<0.01); Initial day versus 6-day (ns).
6) Chl b (w_2). All comparisons significant (p<0.01).
7) Protein (w_0). All comparisons significant (p<0.01).
8) Protein (w_1). All but one comparisons significant (p<0.01); Initial day versus 6-day (ns).
9) Protein (w_2). Initial day versus 9-day significant (p<0.01)
   3-day versus 9-day significant (p<0.01)
   6-day versus 9-day significant (p<0.01)
   Initial day versus 3 day ns.
   Initial day versus 6-day ns.
   3-day versus 6-day ns.
10) Protease activity (w_0). All but two comparisons significant (p<0.01); Initial day versus 9-day (ns); 3-day versus 6-day (ns).
11) Protease activity (w_1). All but two comparisons significant (p<0.01); Initial versus 3-day (ns); 3-day versus 6-day (ns).
12) Protease activity (w_2). All but one comparisons significant.
13) RNA (w_0). All comparisons significant (p<0.01).
14) RNA (w_1). All but one comparisons significant (p<0.01); Initial day versus 3-day (ns).
15) RNA (w_2). All but one comparisons significant (p<0.01); Initial day versus 3-day (ns).
16) DNA (w_0). All comparisons significant (p<0.01).
17) DNA (w_1). All comparisons significant (p<0.01).
18) DNA (w_2). All comparisons non-significant.

Treatment wise

1) Chl a (3-day). All but one comparisons significant (p<0.01); w_0 vs w_1 (ns).
2) Chl a (6-day). w_0 versus w_1 significant (p<0.01)
   w_0 versus w_2 significant (p<0.01)
   w_1 versus w_2 ns.
3) Chl a (9-day). All comparisons ns.
4) Chl b (3-day). All comparisons ns.
5) Chl b (6-day). All comparisons ns.
6) Chl b (9-day). All but one comparisons significant (p<0.05); w_0 versus w_1 (ns).
7) Protein (3-day). w_0 versus w_2 significant (p<0.01)
   w_1 versus w_2 significant (p<0.05)
   w_0 versus w_1 ns.
8) Protein (6-day). w_0 versus w_2 significant (p<0.01)
   w_1 versus w_2 significant (p<0.05)
   w_0 versus w_1 ns.
9) Protein (9-day). All comparisons significant (p<0.01)
10) Protease activity (3-day). All but one comparisons significant (p<0.01); w_1 versus w_2 (ns).
11) Protease activity (6-day). All comparisons significant.
   w_0 versus w_1 significant (p<0.05)
   w_0 versus w_2 significant (p<0.01)
   w_1 versus w_2 significant (p<0.01)
12) Protease activity (9-day). All comparisons significant (p<0.01).
13) RNA (3-day). All but one comparisons significant (p<0.01). w_1 versus w_2 (ns).
14) RNA (6-day). All comparisons (ns).
15) RNA (9-day). All comparisons significant (p<0.01).
16) DNA (3-day). All comparisons (ns).
17) DNA (6-day). All comparisons (ns).
18) DNA (9-day). All comparisons (ns).

Table 7. *C. cajan* leaf discs showing changes in chlorophyll contents (mg g^{-1} fr. wt. ± S.E.), protein (mg/100 mg dry wt. ± S.E.), protease activity (n moles of tyrosine g^{-1} hr^{-1} fr. wt. ± S.E.), RNA and DNA (µg g^{-1} dry wt. ± S.E.) at 24 h interval under varying degrees of water stress induced by PEG.

Leaf constituents	0-hr	24-hr			48-hr			72-hr		
	S_0	S_0	S_1	S_2	S_0	S_1	S_2	S_0	S_1	S_2
Chl-a	2.202 ±0.10	1.990 ±0.21	1.624 ±0.01	1.401 ±0.01	1.409 ±0.10	0.992 ±0.09	0.952 ±0.03	0.872 ±0.01	0.608 ±0.01	0.532 ±0.01
Chl-b	0.354 ±0.01	0.472 ±0.01	0.532 ±001	0.528 ±0.01	0.580 ±0.01	0.672 ±0.03	0.624 ±0.01	0.598 ±0.012	0.664 ±0.030	0.692 ±0.01
Total Chl	2.556 ±0.11	2.462 ±0.22	2.156 ±0.011	1.929 ±0.02	1.989 ±0.11	1.664 ±0.12	1.576 ±0.04	1.470 ±0.022	1.272 ±0.04	1.224 ±0.02
Chl a:b	6.22	4.21	3.05	2.65	2.42	1.47	1.52	1.45	0.91	0.76
Protein	6.82 ±0.11	6.04 ±0.09	5.84 ±0.03	5.32 ±0.01	4.32 ±0.011	3.72 ±0.09	3.50 ±0.11	3.02 ±0.09	2.42 0.12	2.08 ±0.01
Protease activity	2880 ±84.0	3472 ±84.0	3780 ±102.0	3842 ±98.0	4408 ±98.0	4946 ±72.0	5072 ±102.0	5492 ±142.0	5931 ±142.0	6154 ±160.0
RNA	0.272 ±0.001	0.230 ±0.001	0.212 ±0.001	0.201 ±0.003	0.170 ±0.003	0.143 ±0.003	0.128 ±0.005	0.120 ±0.002	0.101 ±0.001	0.089 ±0.003
DNA	0.068 ±0.001	0.058 ±0.003	0.048 ±0.005	0.044 ±0.001	0.041 ±0.003	0.032 ±0.001	0.032 ±0.003	0.030 ±0.001	0.021 ±0.003	0.018 ±0.001
RNA : DNA	4.00	3.96	4.41	4.56	4.14	4.46	4.00	4.00	4.80	4.96

S_0 = Control; S_1 = 11.5%; PEG; S_2 = 23.5% PEG.

Hour wise

1) Chl a (S_0). All but one comparisons significant.
   0h versus 48h significant (p<0.01);
   0h versus 72h significant (p<0.01).
   24 h versus 48h significant (p<0.05);
   24h versus 72h significant (p<0.01).
   48 h versus 72h significant (p<0.05);
2) Chl a (S_1). All comparisons significant (p<0.01).
3) Chl a (S_2). All comparisons significant (p<0.01).
4) Chl b (S_0). All but one comparisons significant (p<0.01). 48h versus 72h (ns).
5) Chl b (S_1). All but one comparisons significant (p<0.01). 48 h versus 72 h (ns).
6) Chl a (S_2). All comparisons significant (p<0.01).
7) Protein (S_0). All comparisons significant (p<0.01).
8) Protein (S_1). All comparisons significant (p<0.01).
9) Protein (S_2). All comparisons significant (p<0.01).
10) Protease activity (S_0). All comparisons significant (p<0.01).
11) Protease activity (S_1). All comparisons significant (p<0.01).
12) Protease activity (S_2). All comparisons significant (p<0.01).
13) RNA (S_0). All comparisons significant (p<0.01).
14) RNA (S_1). All comparisons significant (p<0.01).
15) RNA (S_2). All comparisons significant (p<0.01).
16) DNA (S_0). All comparisons significant (p<0.01).
17) DNA (S_1). All comparisons significant
    0h versus 24h significant (p<0.01)
    0h versus 48h significant (p<0.01).
    0h versus 72h significant (p<0.01).
    24 h versus 48h significant (p<0.05).
    24 h versus 72h significant (p<0.01).
    48 h versus 72h significant (p<0.05).
18) DNA (S_2). All comparisons significant (p<0.01)

Treatment wise

1) Chl a (24h). Only one comparison significant (p<0.01); S_0 versus S_2 significant.
2) Chl a (48h). All but one comparison significant (p<0.01); S_1 versus S_2 (ns).
3) Chl a (72h). All comparisons significant (p<0.01).
4) Chl b (24h). All but one comparison significant (p<0.01); S_1 versus S_2 (ns).
5) Chl b (48h). Only one comparison significant (p<0.01); S_0 versus S_1 significant.
6) Chl b (72h). S_0 versus S_1 significant (p<0.05)
   S_0 versus S_2 significant (p<0.01)
   S_1 versus S_2 (ns)
7) Protein (24h). S_0 versus S_1 significant (p<0.05)
   S_0 versus S_2 significant (p<0.01)
   S_1 versus S_2 significant (p<0.01)
8) Protein (48 h). All but one comparisons significant (p<0.01). S_1 versus S_2 (ns).
9) Protein (72 h). S_0 versus S_1 significant (p<0.01)
S_0 versus S_2 significant (p<0.01)
S_1 versus S_2 significant (p<0.05)
10) Protease (24 h). Only one comparison significant (p<0.05); S_1 versus S_2 significant.
11) Protease (48 h). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
12) Protease (72 h). Only one comparisons significant (p<0.05); S_0 versus S_2 significant.
13) RNA (24h). All comparisons significant (p<0.01).
14) RNA (48h). All comparisons significant.
    S_0 versus S_1 significant (p<0.01)
    S_0 versus S_2 significant (p<0.01)
    S_1 versus S_2 significant (p<0.05)
15) RNA (72h). All comparisons significant (p<0.01).
16) DNA (24h). All but one comparisons significant (p<0.01); S_1 versus S_2 (ns).
17) DNA (48h). All but one comparisons significant (p<0.01). S_1 versus S_2 (ns).
18) DNA (72h). All comparisons significant (p<0.01).

Table 8. *C. cajan* leaf discs showing changes in chlorophyll contents (mg g^{-1} fr. wt. ± S.E.), protein (mg/100 mg dry wt. ± S.E.), protease activity (n moles of tyrosine g^{-1} hr^{-1} fr. wt. ± S.E.), RNA and DNA (µg g^{-1} dry wt. ± S.E.) at 24-hr interval under different combinations of PEG and Kn.

Leaf constituents	0-hr	24-hr			48-hr			72-hr		
	Control	Control	S_x	S_y	Control	S_x	S_y	Control	S_x	S_y
Chl-a	2.184 ±0.01	1.772 ±0.10	2.061 ±0.01	1.908 ±0.10	1.292 ±0.03	1.618 ±0.01	1.474 ±0.03	0.611 ±0.01	0.703 ±0.012	0.664 ±0.03
Chl-b	0.362 ±0.10	0.408 ±0.01	±0.382 ±0.01	0.398 ±0.10	0.592 ±0.01	0.464 ±0.10	0.528 ±0.09	0.601 ±0.01	0.554 ±0.01	0.590 ±0.001
Total Chl	2.546 ±0.11	2.180 ±0.11	2.443 ±0.02	2.306 ±0.20	1.884 ±0.04	2.082 ±0.11	2.002 ±0.12	1.211 ±0.02	1.257 ±0.02	1.254 ±0.03
Chl a:b	6.03	4.34	5.39	4.79	2.18	3.48	2.79	1.01	1.26	1.12
Protein	6.08 ±0.09	5.20 ±0.11	5.59 ±0.09	5.32 ±0.11	3.82 ±0.10	4.48 ±0.09	4.16 ±0.10	2.92 ±0.01	3.94 ±0.09	3.36 ±0.09
Protease activity	2942 ±102	3698 ±102	34.60 ±98	3698 ±102	4490 ±84	4002 ±112	4128 ±92	5672 ±121	5012 ±98	5298 ±142
RNA	0.254 ±0.003	0.202 ±0.001	0.218 ±0.003	0.204 ±0.003	0.152 ±0.003	0.174 ±0.003	0.162 ±0.001	0.101 ±0.001	0.138 ±0.002	0.121 ±0.001
DNA	0.058 ±0.001	0.051 ±001	0.054 ±0.003	0.051 ±0.003	0.028 ±0.001	0.040 ±0.001	0.034 ±0.001	0.020 ±0.003	0.031 ±0.001	0.023 ±0.002
RNA : DNA	4.37	3.96	4.03	4.00	5.42	4.35	4.76	5.05	4.45	5.26

Control = Distilled water; Sx = 11.5% PEG + 0.05 mM Kn; Sy = 23.5% PEG + 0.05mM Kn.

Hour wise

1) Chl a (Control). All comparisons significant (p<0.01).
2) Chl a (Sx). All comparisons significant (p<0.01).
3) Chl a (Sy). All comparisons significant (p<0.01).
4) Chl b (Control). 0h versus 24 h ns, 0h versus 48 h significant (p<0.05).
   0h versus 72 h significant (p<0.05), 24 h vs 48 h ns,
   24 h versus 72 h significant (p<0.05), 48 h vs 72 h ns.
5) Chl b (Sx). All comparisons ns.
6) Chl b (Sy). All comparisons ns.
7) Protein (Control). All comparisons significant (p<0.01)
8) Protein (Sx). All comparisons significant (p<0.01)
9) Protein (Sy). All comparisons significant (p<0.01)
10) Protease activity (Control). All comparisons significant (p<0.01)
11) Protease activity (Sx). All comparisons significant (p<0.01)
12) Protease activity (Sy). All comparisons significant; 24 h versus 48 h significant at P<.05; Rest comparisons significant at p<0.01.
13) RNA (Control). All comparisons significant (p<0.01)
14) RNA (Sx). All comparisons significant (p<0.01)
15) RNA (Sy). All comparisons significant (p<0.01)
16) DNA (Control). All but two comparisons significant (p<0.01); 0h versus 24 h (ns); 48 h versus 72 h (ns).
17) DNA (Sx). All but two comparisons significant (p<0.01); oh versus 24 h (ns); 48 h versus 72 h (ns).
18) DNA (Sy). All but one comparisons significant (p<0.01); oh versus 24 h (ns);

Treatment wise

1) Chl a (24 h). All comparisons ns.
2) Chl a (48 h). All comparisons significant (p<0.01).
3) Chl a (72 h). Only one comparison significant (p<0.01); control versus Sx significant.
4) Chl b (24 h). All comparisons ns.
5) Chl b (48 h). All comparisons ns.
6) Chl b (72 h). Only one comparison significant (p<0.05); control versus Sx significant.
7) Protein (24 h). Only one comparison significant (p<0.05); Control versus Sx significant.
8) Protein (48 h). Control versus Sx significant (p<0.01); Control versus Sy significant (p<0.01), Sx versus Sy ns.
9) Protein (72 h). All comparisons significant (p<0.01).
10) Protease activity (24 h). All comparisons ns.
11) Protease activity (48 h). Control versus Sx significant (p<0.01).
   Control versus Sy significant (p<0.01).
   Sx versus Sy ns.
12) Protease activity (72 h). Control one comparison significant (p<0.01); Control versus Sx significant.
13) RNA (24 h). All but one comparison significant (p<0.01); Control versus Sy (ns).
14) RNA (48 h). All comparisons significant (p<0.01).
15) RNA (72 h). All comparisons significant (p<0.01).
16) DNA (24 h). All comparisons ns (p<0.01).
17) DNA (48 h). Control versus Sx significant (p<0.01).
   Control versus Sy significant (p<0.05).
   Sx versus Sy significant (p<0.05).
18) DNA (72 h).
   Control versus Sx significant (p<0.01).
   Control versus Sy significant (p<0.05).
   Sx versus Sy significant (p<0.05).

The relative increase in chl b content was found to be more than that of chl a after first three days of treatment (Supp. Fig. 4, 5). The relative changes in leaves at 6 and 9-day stages of seedlings were also comparatively less negative in case of chl b than chl a. (Supp. Fig. 4, 5). PEG treatment reduced the magnitude of negative changes in chl b at 6 and 9-day stages (Supp. Fig. 5). The relative change in chl a : b ratio showed maximum negative value at 6-day after Kn treatment (Supp. Fig. 7) when compared to PEG, and also PEG + Kn treatment, showing the maximum negative change at 9-day (Supp. Fig. 7).

The relative changes in protein showed positive values at 3-day and negative values thereafter (Supp. Fig. 8). The relative change trends were strikingly different in case of protease activity after Kn applications with positive values at all stages when compared to PEG and PEG + Kn treatments, with 3-day stage showing negative values and later stages showing positive values (Supp. Fig. 9). Both in RNA and DNA, the relative changes assumed positive values at 3-day and became negative at 6 and 9-day stages (Supp. Fig. 10, 11). High negative changes were observed in RNA : DNA ratio at 6-day stage (Supp. Fig. 12).

Experiments with pigeonpea leaf discs also indicated that stress effects follow almost similar pattern as witnessed in seedling studies. Both chl a and chl b decreased gradually during 72 h in unstressed and stressed leaf discs; the decline was much greater in stressed sets. It was really interesting to note a sharp decline in chl a : chl b ratio due to rapid fall in the amount of chl a; specially under water stress. The magnitude of decline in chl b was much smaller as compared to chl a. The fall in the chlorophyll content was associated with the onset of senescence. Panigrahi and Biswal (1979) also found the decline in total chl after full expansion of leaves.

In leaf disc experiments, the relative change values were negative all along for chl a (Supp. Fig. 13), with maximum negative value observed at 72 h stage in Kn + PEG treatment. The relative change trends were completely different in case of chl b with positive values all along (Supp. Fig. 14). In PEG treatment, maximum positive values were observed at 48 h stage. The relative change values in total chl and chl a:b ratio were found to be negative throughout (Supp. Fig. 15, 16).

Amounts of protein, RNA and DNA also declined considerably by PEG treatments (Fig. 17a, 19a, 20a). On the other hand, Kn treatment was able to neutralize partly the stressed effects, specially with 11.5% PEG concentration as evident from the amount of protein and nucleic acids (Fig. 17b, 19b, 20b). Kn was also able to minimize the protease activity (Fig. 18 a-b). The relative changes for protein were negative while that for protease were positive all along (Supp. Fig. 17, 18). These relative changes were negative at all stages for RNA and DNA (Supp. Fig. 19, 20). The trend of relative change was similar in PEG treatment and also PEG + Kn treatment at 72 h stage, for RNA : DNA ratio whereas trends got reversed under these two treatments at 24 h and 48 h stages (Supp. Fig. 21).

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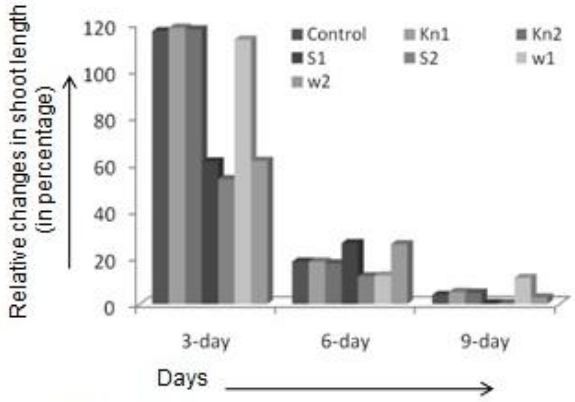
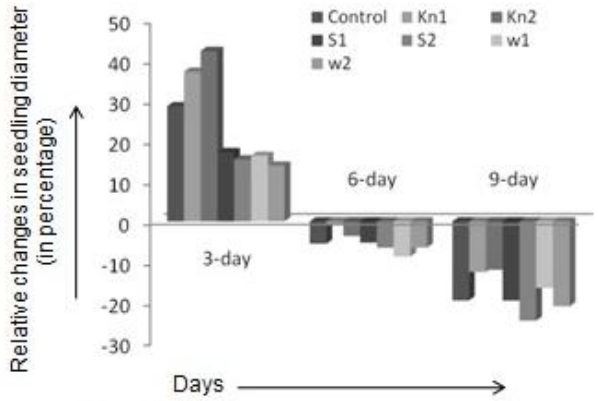
APPENDICES

SUPPLEMENTARY FIGURES



Supp. Fig. 1



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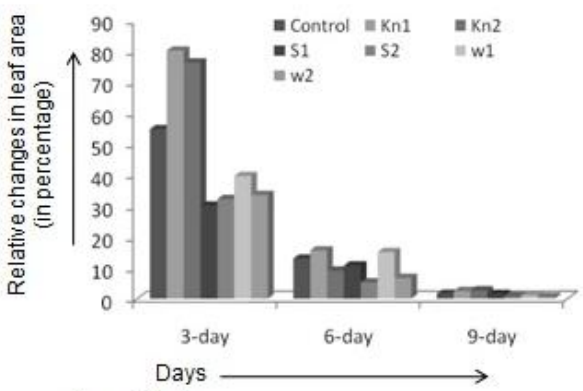

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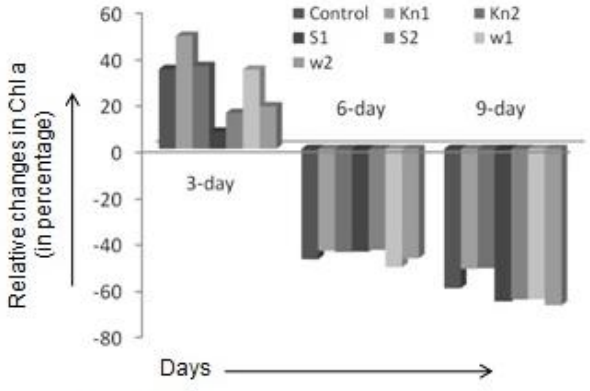


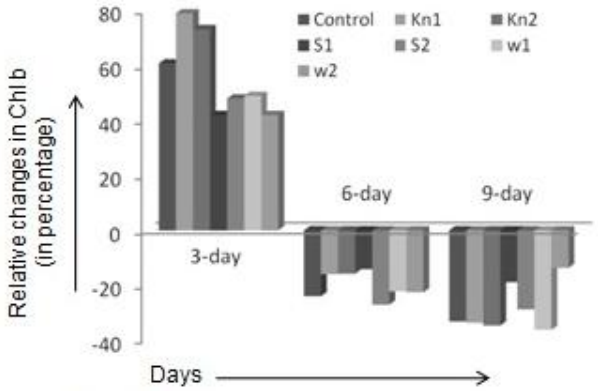
Supp. Fig. 4

Supplementary Fig. 1: *Cajanus cajan* seedlings showing relative changes in shoot length (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 2: *C. cajan* seedlings showing relative changes in diameter (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 3: *C. cajan* seedlings showing relative changes in leaf area (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 4: *C. cajan* seedlings showing relative changes in Chl a of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.



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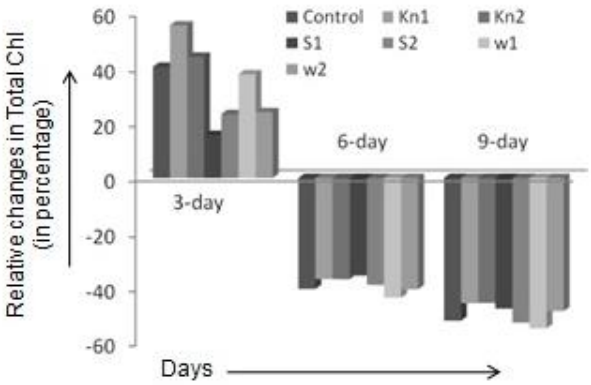

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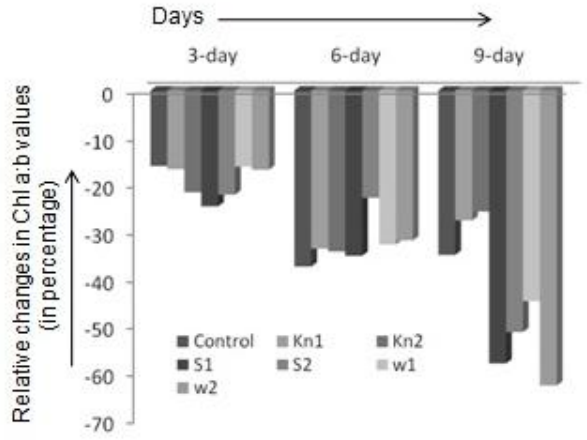

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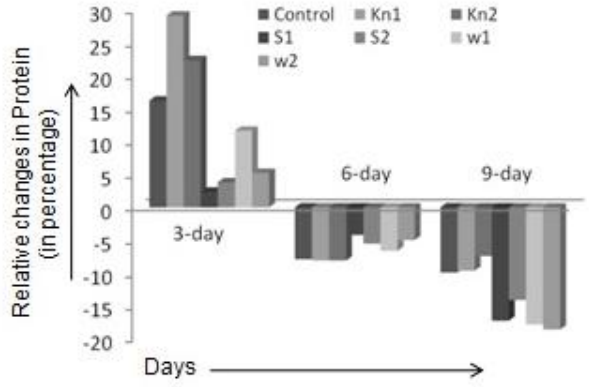


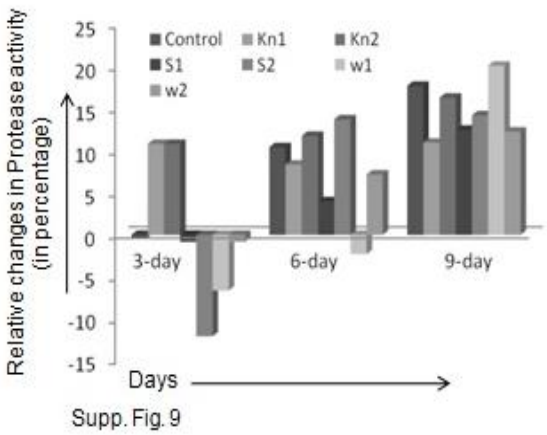
Supp. Fig. 8

Supplementary Fig. 5: *C. cajan* seedlings showing relative changes in Chl b of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 6: *C. cajan* seedlings showing relative changes in Total Chl of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 7: *C. cajan* seedlings showing relative changes in Chl a:b ratio of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 8: *C. cajan* seedlings showing relative changes in protein-content of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.



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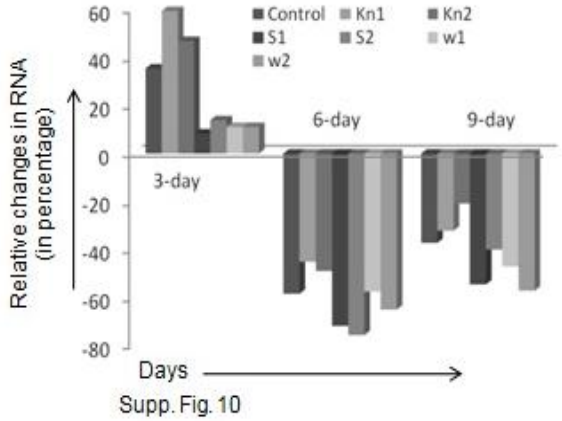

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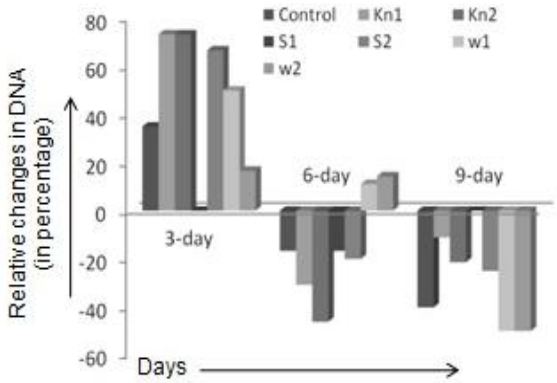

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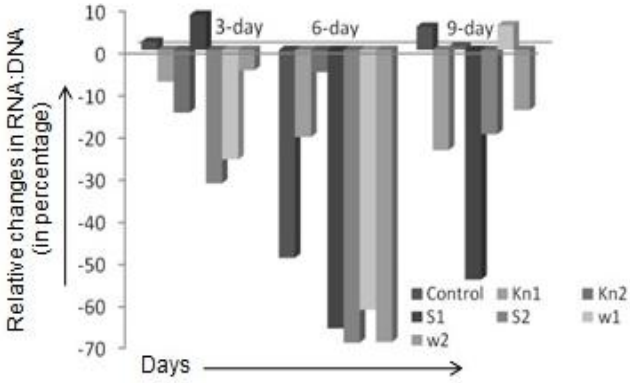


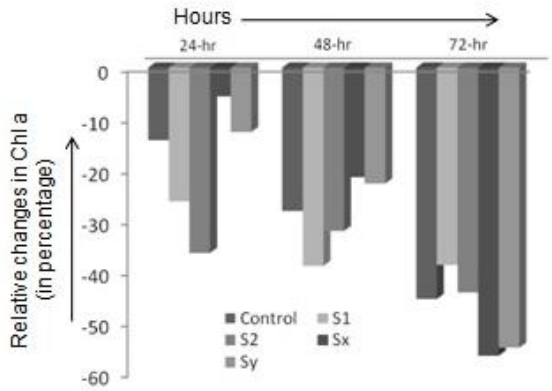
Supp. Fig. 12

Supplementary Fig. 9: *C. cajan* seedlings showing relative changes in protease activity of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 10: *C. cajan* seedlings showing relative changes in RNA-content of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 11: *C. cajan* seedlings showing relative changes in DNA-content of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.

Supplementary Fig. 12: *C. cajan* seedlings showing relative changes in RNA:DNA ratio of leaves (in percentage) under Kn (Kn1 and Kn2) treatments, PEG (S1 and S2) treatments and, Kn + PEG (w1 and w2) treatments. For each day, the order, in which bars are arranged on the graph, is Control, Kn1, Kn2, S1, S2, w1 and w2, respectively.



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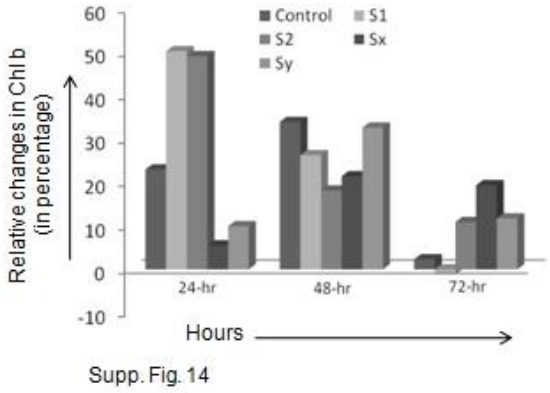

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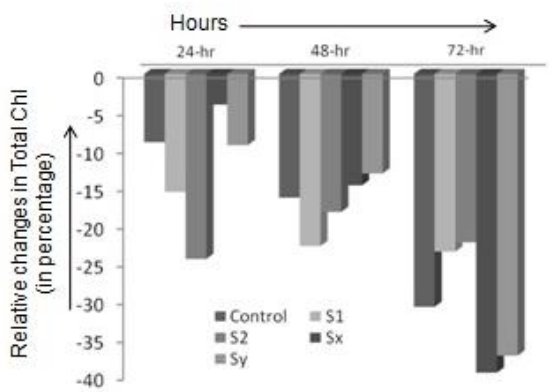

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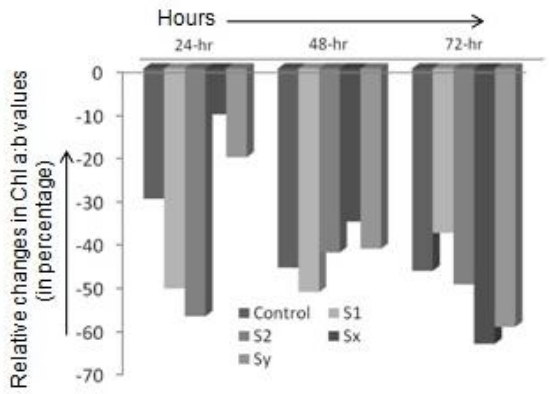


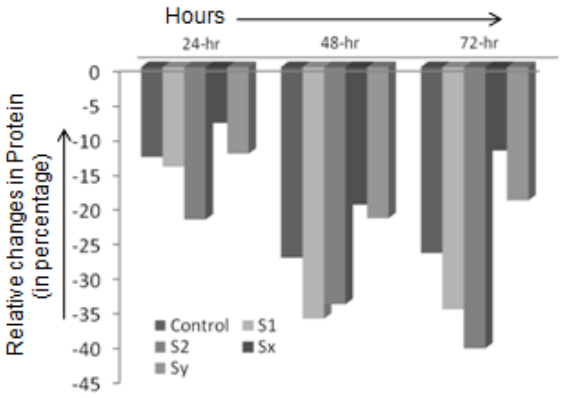
Supp. Fig. 16

Supplementary Fig. 13: *C. cajan* leaf discs showing relative changes in Chl a, measured in percentage, under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.

Supplementary Fig. 14: *C. cajan* leaf discs showing relative changes in Chl b, measured in percentage, under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively..

Supplementary Fig. 15: *C. cajan* leaf discs showing relative changes in Total Chl, measured in percentage, under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.

Supplementary Fig. 16: *C. cajan* leaf discs showing relative changes in Chl a:b ratio, measured in percentage, under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.



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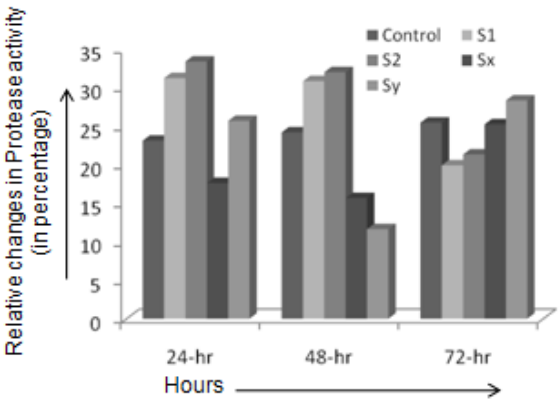

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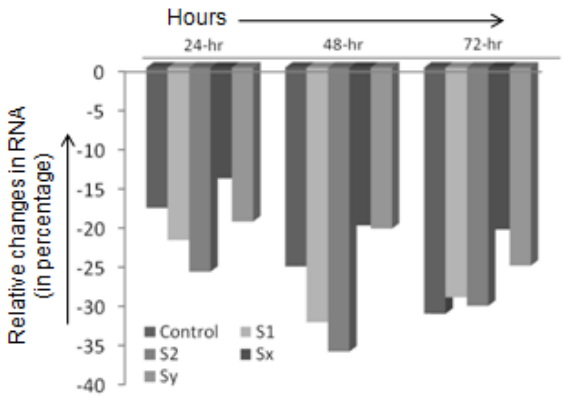

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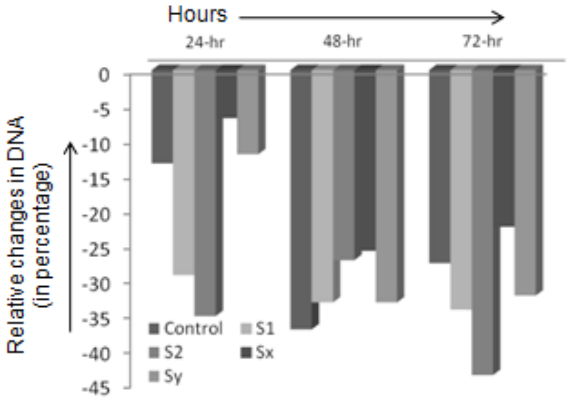


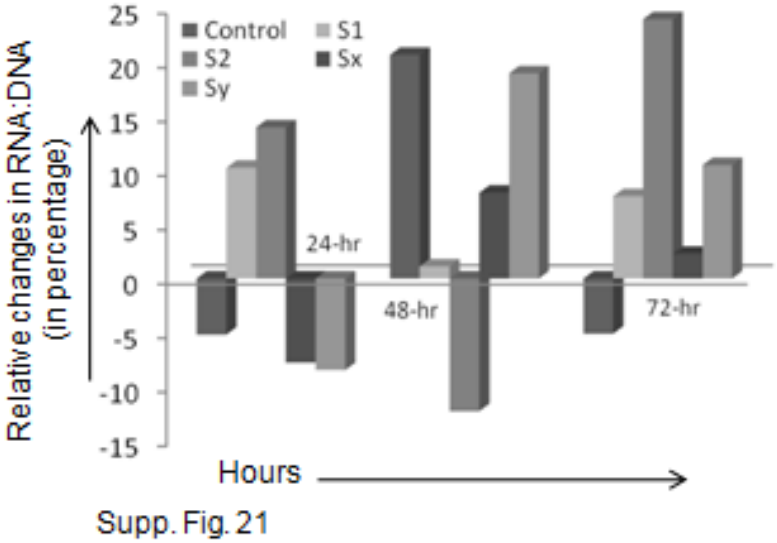
Supp. Fig. 20

Supplementary Fig. 17: *C. cajan* leaf discs showing relative changes in protein-content (measured in percentage) under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.

Supplementary Fig. 18: *C. cajan* leaf discs showing relative changes in protease activity (measured in percentage) under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.

Supplementary Fig. 19: *C. cajan* leaf discs showing relative changes in RNA-content (measured in percentage) under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.

Supplementary Fig. 20: *C. cajan* leaf discs showing relative changes in DNA-content (measured in percentage) under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.



Supplementary Fig. 21: *C. cajan* leaf discs showing relative changes in RNA:DNA ratio (measured in percentage) under PEG (S1 and S2) treatments and, Kn + PEG (Sx and Sy) treatments. For each hour, the order, in which bars are arranged on the graph, is Control, S1, S2, Sx and Sy, respectively.