

BRIEF COMMUNICATION

Amelioration of negative effect of water stress in *Cassia angustifolia* by benzyladenine and/or ascorbic acid

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The effect of benzyladenine (BA) and ascorbic acid (AA) on relative water content, proline accumulation, net photosynthetic rate (P_N), chlorophyll (Chl) content and nitrate reductase (NR) activity, under sufficient water supply and moisture stress was studied in senna (*Cassia angustifolia* Vahl.) at seedling, vegetative, flowering and pod formation stages. AA treatment resulted in a higher accumulation of proline at all the stages of growth. Both BA and AA enhanced P_N , Chl content and NR activity, and ameliorate the negative effect of water stress.

Additional key words: chlorophyll content, nitrate reductase activity, photosynthetic rate, proline content, relative water content, senna.

Senna (*Cassia angustifolia*) an important medicinal plant, is widely known for its sennoside content which is used to cure a large number of intestinal diseases, jaundice, anaemia, typhoid fever and skin diseases. Senna suffers from early senescence and moisture stress further aggravates the situation.

Chinoy *et al.* (1969) reported that ascorbic acid (AA) is beneficial for barley growth and development under water stress. Krishnappa and Murty (1979) observed that AA increased the yield in rice. In soybean, Dong *et al.* (1995) reported that benzyladenine (BA) increased the chlorophyll contents, photosynthetic rate and Rubisco activity under moisture stress. With these results in mind the present experiments were taken to find if AA and/or BA ameliorate negative effect of moisture stress in senna.

Seeds of senna (*Cassia angustifolia* Vahl.), obtained from Gujrat Agricultural University, Anand, Gujrat (India), were sown in the first week of July 1997 in pots (10 seed per pot) filled with soil and *Farm Yard Manure* in ratio of 4:1. The basal dose of NPK was applied at the time of pot filling and at vegetative stage before giving treatments. Five plants per pot were maintained at

seedling stage and four plants at later stages. Before stress treatment, BA solution (20 mg dm^{-3}) was sprayed on leaves, while AA was given into soil (1.7 mg kg^{-1}). A four-day desiccation cycle was given by withholding the water supply at four phenological stages, *i.e.*, seedling (20 DAS), vegetative (50 DAS), flowering (70 DAS) and pod formation (90 DAS) stages. Plants were re-watered on the termination of the desiccation cycle. Control plants were watered regularly. Relative water content of leaves was determined by the method of Barrs and Weatherley (1962). Net photosynthetic rate was recorded with photosynthesis system (*Licor 6100*, Lincoln, USA) between 10:00 and 11:00. Total chlorophyll content was measured following the method of Hiscox *et al.* (1979): leaves were extracted by dimethylsulphoxide, kept at 65°C for 3 h, and the absorbance of supernatant was recorded at 645 and 663 nm on spectrophotometer *Spectronic-20* (*Bausch and Lomb*, New York, USA) For determination of nitrate reductase activity fresh fully expanded leaves were cut into small pieces (0.5 g) and kept in 2.5 cm^3 of 0.1 M phosphate buffer (pH 7.5) and 2.5 cm^3 of 0.4 M KNO_3 . Infiltration of leaf tissue was

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Abbreviations: AA - ascorbic acid; BA - benzyladenine; Chl - chlorophyll; DAS - days after sowing; NR - nitrate reductase; P_N - net photosynthetic rate; RWC - relative water content.

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done for 2 min. Tubes were then kept for 30 min in water bath at 30 °C for incubation. The reaction was stopped by transferring tubes in boiling water and then cooled at room temperature. Assay was done following the method of Hageman *et al.* (1971). Proline content was estimated in fully expanded leaves according to Bates *et al.* (1973). Fresh cleaned leaves (0.5 g) were homogenised in 10 cm³

of 3 % sulfosalicylic acid and centrifuged at 22 000 g for 5 min. 2 cm³ of supernatant, 2 cm³ of acid ninhydrin and 2 cm³ of glacial acetic acid were mixed. The mixture was boiled at 100 °C in water bath for 1 h, and further extracted in 10 cm³ of toluene by mixing thoroughly in test tube. The absorbance was measured at 520 nm. L-proline (*Sigma*, St. Louis, USA) was used as standard.

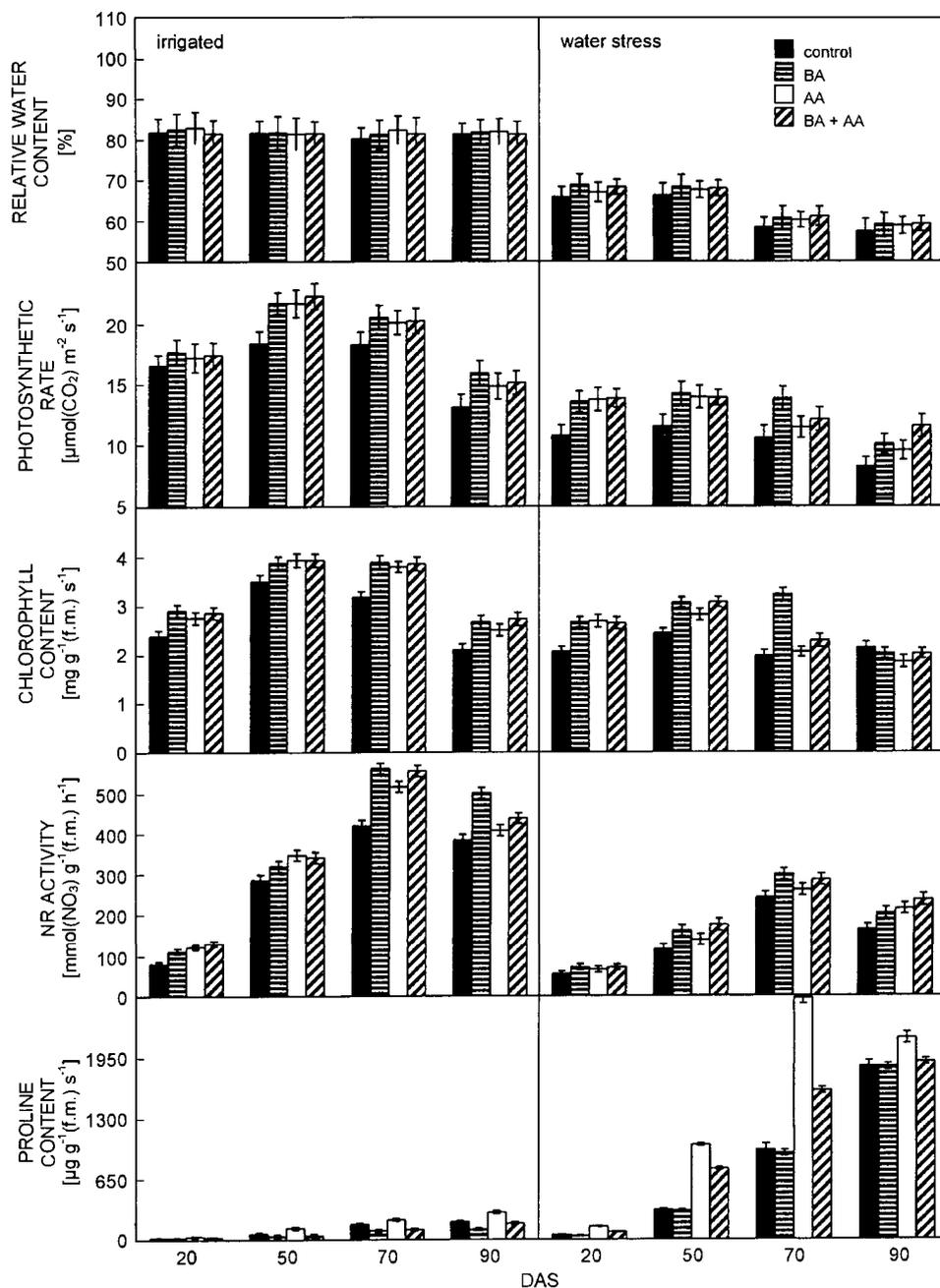


Fig. 1. Effect of benzyladenine (BA) and ascorbic acid (AA) on relative water content, net photosynthetic rate, chlorophyll content, nitrate reductase (NR) activity, and proline content in irrigated (control) plants and plants water stressed for 4 d during seedling (20 DAS), vegetative (50 DAS), flowering (70 DAS), or pod formation (90 DAS) growth stages.

The physiological parameters were recorded at different phenological stages and the basis for taking observation was the visual symptoms of initiation of leaf wilting.

Relative water content (RWC) measured in the upper fully expanded leaf ranged from 65 - 68 % at seedling and vegetative stages, 55 - 60 % at later stages under stress while 80 - 82 % under sufficient irrigation. No effects of BA, AA and their combination on RWC was observed.

Water stress decreased photosynthetic rate (P_N). BA and AA either alone or in combination, enhanced the P_N under sufficient water supply (Fig. 1). The difference between BA and AA effect were not so conspicuous. Effects of BA and AA either alone or in combination on chlorophyll (Chl) content in leaves were similar as noted for P_N . These results agree with work of Dong *et al.* (1995) who reported that foliar spray of BA increased P_N and Chl content in maize seedling under soil drought. AA and BA applied individually or in combination resulted in a higher nitrate reductase (NR) activity at all the growth stages under moisture stress conditions. BA enhanced the NR activity a little more as compared to AA treatment especially under moisture stress (Fig. 1). Our results are in agreement with those of Gzik *et al.* (1987) who reported that kinetin and BA stimulated NR activity in

sugar beet leaves under water stress. AA increased more than two folds proline content at seedling stage, and at later stages almost three-fold increase was noted (Fig. 1). Several workers have studied the effect of moisture stress on proline content, *e.g.*, Singh *et al.* (1972) in barley leaves. Fedina and Popova (1996) found that proline accumulation in pea leaves increased as RWC decreased. Stewart and Hanson (1980) suggested that accumulation of proline represented the adaptation to water stress. Proline accumulation has been correlated with tolerance not only to drought but also to salinity in plants (*e.g.*, Kishor *et al.* 1995). Prabha and Bharti (1980) have shown that proline counteracts the effect of water stress and AA treatment makes plants more resistant to water stress. Chinoy *et al.* (1969) had reported a beneficial effect of AA under water stress as well as under irrigated conditions in barley. In the present experiment proline accumulation was found very high due to AA treatment. It may be argued that enhanced proline synthesis occurs under stress conditions and ascorbic acid further enhances its synthesis and helps plant to withstand the stress. Aspinall *et al.* (1981) reported that water stress induced accumulation of proline in wheat coleoptile which was further stimulated by zeatin.

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