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BRIEF COMMUNICATION

Carbendazim alleviates effects of water stress on chickpea seedlings

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Abstract

Carbendazim (methyl-2-benzimidazole carbamate) promoted root growth of chickpea (*Cicer arietinum* L.) seedlings subjected to polyethylene glycol (PEG, osmotic potential -0.5 MPa) induced water stress. The relative water content, membrane stability index, 2,3,5-triphenyltetrazolium chloride reduction and contents of some osmolytes (proline, sucrose, glucose and fructose) enhanced significantly while the contents of lipid peroxides and hydrogen peroxide diminished effectively by addition of 0.05 % carbendazim into PEG solution.

Additional key words: *Cicer arietinum*, hydrogen peroxide, malondialdehyde, membrane stability index, proline, root growth.

Carbendazim (methyl-2-benzimidazole carbamate) commercially named *Bavistin* is known as a fungicide against moulds, rots and blight. While using this fungicide to counter the persistent fungal infections in our germination experiments on polyethylene glycol-6000 (PEG) induced water stress in chickpea seeds, we found that carbendazim application not only prevented the fungal infection but also improved the performance of seeds under stress conditions. Previously, fungicides, especially belonging to triazole group (paclobutrazol, uniconizole, triadimefon) have been reported to impart protection against various abiotic stresses (Fletcher *et al.* 2000). The protective action of triazoles has been related to the stimulation of ABA synthesis (Asare-Boamah and Fletcher 1986), enhancement of free radical scavenging capacity by increasing the content of superoxide dismutase, catalase, peroxidase (Feng *et al.* 2003), and ascorbate peroxidase (Kraus *et al.* 1995), and osmoregulation (Muthukumarasamy *et al.* 2000). The present findings report for the first time the protective effect of carbendazim against water stress along with its possible underlying mechanisms in case of chickpea.

Chickpea (*Cicer arietinum* L. cv. Desi GPF2) seeds were surface sterilized with 1 % sodium hypochlorite for 5 min and subsequently washed thoroughly with distilled water. These seeds were grown in Petri dishes in the presence of PEG-6000 (osmotic potential -0.5 MPa; Michel and Kaufmann 1973) alone or in combination with 0.01, 0.05 and 0.10 % carbendazim. This PEG-6000 concentration was opted out of a range -0.3 to -0.8 MPa in a preliminary experiment since it resulted in nearly 50 % reduction in germination and root growth compared to unstressed seeds. Seeds were grown under controlled conditions (day/night temperature of 23/20 °C, 13-h photoperiod; irradiance of 250 μmol m^{-2} s^{-1}). Root growth was recorded at 5-d intervals for 15 d and growth rate was calculated. Electrolyte leakage (EL) test and 2,3,5-triphenyltetrazolium chloride (TTC) reduction assay of stressed roots of 15-d old seedlings were conducted as described by Lutts *et al.* (1996) and Steponkus and Lanphear (1967), respectively. The proline content was estimated by the method of Bates *et al.* (1973) while sucrose, fructose and glucose were analyzed by method of Liu and Van Staden (2001). The contents of ascorbic acid, lipid peroxides (as malondialdehyde) and hydrogen peroxide were estimated according to the methods of Mukherjee and Choudhuri (1983), Heath and Packer (1968) and Teranishi *et al.* (1974), respectively. Sample preparation and H_2O_2 estimation was done as described previously (Nayyar and Kaushal 2002). The experiments were conducted in 3 replications following completely randomized block design, mean values were pooled and standard error (SE) was calculated. *ANOVA* was worked out between the treatments using *SPSS* software.

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Abbreviations: AA - ascorbic acid, MDA - malondialdehyde; MSI - membrane stability index; PEG - polyethylene glycol; RGR - root growth rate; RL - root length; RWC - relative water content; TTC - 2,3,5-triphenyltetrazolium chloride.

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Due to water stress, the root length showed 47 and 63 % reduction over control at 10 and 15 d, respectively (Table 1). With 0.05 % concentration of carbendazim, an increase of 25 and 69 % occurred in root length at 10 and 15 d, respectively while 0.01 and 0.1 % concentrations were ineffective. Root growth rate (RGR) declined by 53 and 87 % over control in stressed seedlings between 5 - 10 and 10 - 15 d, respectively (Table 1). Carbendazim at 0.05 % concentration caused a 47 % increase in RGR between 5 - 10 while 4.5 times increase was observed between 10 - 15 d. Membrane stability index (MSI) and 2,3,5-triphenyl reduction (TTC) activity declined markedly in roots of stressed seedlings (Table 1). MSI increased by 25 and 16 % with 0.05 and 0.1 % carbendazim, respectively while TTC reduction activity was enhanced by 82 and 72 %, respectively with these concentrations. Water stress caused 35 % reduction in relative water content (RWC) compared to control (Table 1). A significant increase (18 %) was noticeable in RWC with 0.05 % carbendazim while 0.1 % concentration resulted in 11 % increase. Among solutes, proline content increased due to stress by 2.5 times over controls (Table 1). The contents of proline increased appreciably with all the concentrations of carbendazim and to a greater extent by 0.05 and 0.1 %. Sucrose, glucose and fructose showed an increase of 37, 66 and 64 %, respectively in stressed seedlings as compared to controls (Table 1). With carbendazim, an additional and marked increase was noticeable, particularly with 0.05 and 0.1 % concentrations. Glucose and fructose were elevated to a relatively higher extent than sucrose. Oxidative damage in terms of malondialdehyde (MDA) content increased by 5 times while H_2O_2 content elevated by 4.5 times in stressed seedlings (Table 1). With 0.05 % carbendazim, MDA content decreased by 20 % while those of H_2O_2 reduced by 17 %. Ascorbic acid content increased significantly in stressed seedlings relative to control (Table 1). In the presence of 0.05 % carbendazim, 22 % increase in ascorbic content occurred in stressed plants.

Table 1. Effect of osmotic stress induced by PEG (osmotic potential of -0.5 MPa) and carbendazim on root length (RL), root growth rate (RGR), membrane stability index (MSI), 2,3,5- triphenylterazolium chloride reduction activity (TTC), root water content (RWC), and accumulation of proline, sucrose, glucose, fructose, malondialdehyde (MDA), H_2O_2, and ascorbic acid (AA) in chickpea seedlings. Observations were mostly taken on 15-d old seedlings. Data represent means ± SE.

Parameter	Control	PEG	PEG + 0.01 % carbendazim	PEG + 0.05 % carbendazim	PEG + 0.1 % carbendazim	C.D. ($P < 0.05$)
RL [cm] 5 d	4.0 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.7 ± 0.2	3.2 ± 0.2	0.3
RL [cm] 10 d	10.7 ± 1.2	5.6 ± 2.2	5.9 ± 2.8	..7.1 ± 2.4	5.0 ± 2.9	2.8
RL [cm] 15 d	15.5 ± 2.1	5.8 ± 2.3	6.1 ± 1.5	9.9 ± 1.7	5.4 ± 1.7	2.6
RGR [cm d^{-1}] 5-10 d	1.3 ± 0.6	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.3
RGR [cm d^{-1}] 10-15 d	0.9 ± 0.2	0.1 ± 0.01	0.04 ± 0.01	0.5 ± 0.1	0.07 ± 0.02	0.5
MSI [%]	87.1 ± 3.4	42.3 ± 2.6	45.8 ± 2.7	67.8 ± 2.4	58.4 ± 3.1	4.2
TTC [ΔA g^{-1}(f.m.)]	2.3 ± 0.8	1.2 ± 0.2	2.0 ± 0.4	2.1 ± 0.2	1.0 ± 0.2	0.7
RWC [%]	86.2 ± 3.2	51.2 ± 2.8	51.6 ± 2.6	69.2 ± 3.1	62.2 ± 2.8	3.4
Proline [μg g^{-1} (d.m.)]	18.2 ± 2.6	46.4 ± 2.2	34.3 ± 2.3	26.7 ± 2.1	29.1 ± 2.4	2.8
Sucrose [mg g^{-1} (d.m.)]	40.4 ± 4.1	55.4 ± 4.3	58.5 ± 4.1	86.6 ± 4.2	74.1 ± 4.4	4.6
Glucose [mg g^{-1} (d.m.)]	15.2 ± 4.6	25.2 ± 5.1	28.1 ± 4.8	40.1 ± 5.2	38.7 ± 4.3	5.6
Fructose [mg g^{-1} (d.m.)]	14.2 ± 3.2	23.3 ± 3.4	26.4 ± 2.3	37.7 ± 2.2	32.3 ± 3.1	3.5
MDA [nmol g^{-1} (f.m.)]	21.2 ± 2.4	90.4 ± 2.5	88.3 ± 2.8	72.2 ± 2.1	83.4 ± 2.5	2.6
H_2O_2 [μmol g^{-1} (f.m.)]	8.2 ± 1.8	46.1 ± 2.4	42.2 ± 2.5	38.4 ± 2.5	41.1 ± 2.1	2.4
AA [μmol g^{-1} (d.m.)]	10.1 ± 2.4	17.6± 2.3	18.5 ± 1.8	21.6 ± 1.6	19.2 ± 1.8	2.1

The present study indicated that carbendazim at 0.05 and 0.1 % concentrations acted as a stress protectant in chickpea seedlings. Improvement in root growth by carbendazim could be related to increased RWC. This might possibly be associated with enhanced accumulation of of osmolytes like proline, sucrose, glucose and fructose (Rathinasabapathi 2000, Nayyar 2003/4). Further investigation would be required to probe the precise effect of carbendazim on biosynthesis and degradation of these solutes. A substantial reduction in electrolyte leakage occurred in carbendazim treated plants while TTC reduction activity increased significantly that pointed towards stability of membranes and cellular respiration, respectively, by carbendazim. This might possibly occur due to suppression of oxidative damage in the carbendazim treated cells as shown by reduced malon-dialdehyde (indicator of membrane lipid peroxidation) and H_2O_2 contents coupled with higher contents of ascorbic acid. A significant reduction in oxidative damage by carbendazim also indicated towards its antioxidative role. In this context, our findings match with some of the earlier ones where fungicides belonging to triazoles group have been reported to impart tolerance against salt, osmotic, oxidative and chilling stress

(Fletcher and Nath 1984, Feng *et al*. 2003). Though, structurally, carbendazim differs from triazoles but its protective effects appeared to resemble triazoles as pointed above. It is also pertinent to mention here that carbendazim had proportionately larger effect on solute accumulation than reduction of oxidative damage suggesting that its stress protective effect might arise from greater involvement in osmotic adjustment.

The present study, thus, provides preliminary evidence in support of carbendazim having a stress protective role, however, elaborative studies would be required to have a better insight about its mechanisms.

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