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INFLUENCE OF PACLOBUTRAZOL AND MOISTURE STRESS CONDITIONING ON DROUGHT SUSCEPTIBILITY IN *ROBINIA PSEUDOACACIA* SEEDLINGS

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THAKUR, P. S., CHAUHAN, S., THAKUR, A. & DHALL, S. P. 2000. Influence of paclobutrazol and moisture stress conditioning on drought susceptibility in *Robinia pseudoacacia* seedlings. *Robinia pseudoacacia* nursery stock was conditioned with a combination of paclobutrazol treatments and 2–3 moisture stress cycle, each of 5 days stress followed by 5 days rehydration. Both moisture stress and paclobutrazol (PP_{333}) conditioning improved water relations, enhanced drought tolerance and decreased the drought injury index of conditioned seedlings over unconditioned seedlings at subsequent water stress. Water stress caused shoot and root dry weight reduction, although reduction was significantly less in paclobutrazol conditioned seedlings. Conditioning treatments maintained significantly higher leaf water potential in conditioned than unconditioned seedlings up to 16 days of water stress. Proline, total amino acid and soluble sugar contents were greater in PP_{333} and moisture stress conditioned seedlings during subsequent water deficit. Drought injury index (*Id*) on the 16th day of water deficit was maximum (69.9%) in unconditioned seedlings, whereas *Id* for PP_{333} and moisture stress conditioned seedlings varied between 23 and 36%.

Key words: Paclobutrazol - *Robinia pseudoacacia* - moisture stress conditioning - stress cycles - water potential - drought susceptibility - injury index

THAKUR, P. S., CHAUHAN, S., THAKUR, A. & DHALL, S. P. 2000. Pengaruh paklobutrazol dan penyesuaian tegasan air terhadap kerentanan kemarau dalam anak benih *Robina pseudoacacia*. Stok semaian *Robinia pseudoacacia* disesuaikan dengan gabungan rawatan paklobutrazol dan 2–3 kitaran tegasan air, setiap satu lima hari tegasan diikuti dengan lima hari rehidratan. Kedua-dua penyesuaian tegasan air dan paklobutrazol (PP_{333}) memperbaik kaitan air, meningkatkan tolerans kemarau dan mengurangkan indeks kecederaan kemarau bagi anak benih yang disesuaikan melalui anak benih yang tidak disesuaikan pada tegasan air yang berikutnya. Tegasan air menyebabkan pengurangan berat kering pucuk dan akar, walaupun pengurangan adalah berkurangan dengan bererti dalam anak benih yang disesuaikan dengan paklobutrazol. Rawatan penyesuaian mengekalkan potensi air daun yang lebih tinggi dengan bererti dalam anak benih yang disesuaikan berbanding dengan anak benih yang tidak disesuaikan sehinggalah 16 hari daripada tegasan air. Prolina, jumlah asid amino dan kandungan gula terlarut adalah lebih besar dalam PP_{333} dan anak benih yang disesuaikan dengan tegasan air semasa kekurangan air secara berturut-turut. Indeks kecederaan kemarau (*Ik*) pada hari ke-16 kekurangan air adalah maksimum (69.9%) dalam anak benih yang tidak disesuaikan, manakala *Ik* bagi PP_{333} dan anak benih yang disesuaikan dengan tegasan air berubah antara 23 hingga 36%.

Introduction

Poor establishment and high mortality of transplanted nursery stock cause major setbacks to afforestation programmes. Tree seedlings often have small and underdeveloped root systems at planting, restricting water uptake. Water stress at any developmental phase can be deleterious, affecting almost every aspect of plant growth and development (Clark & Hiller 1973, Cohen & Cohen 1983, Thakur & Thakur 1993, Thakur 1994). Reports are available investigating the detrimental effects of internal water deficits on vital physiological processes in woody plants (Kozlowski 1985, Hallgren & Helms 1988, Parker & Pallardy 1988, Seiler & Cazel 1990, Thakur 1991). Unfortunately, there is little information as to the adaptation strategies of seedlings to tolerate transplanting shock at planting sites. Reports indicate that hardened seedlings are better able to tolerate water stress conditions (Seiler & Johnson 1984, Zwiazek & Blake 1989, Ranney *et al.* 1990, Thakur 1994). Paclobutrazol (PP_{333}), a synthetic growth regulator, has been reported to improve water relations of woody plants (Atkinson & Chauhan 1987, Driessche 1989).

Drought tolerance of seedlings at planting time is an important factor influencing their survival and growth. However, the amount of information on the impact of conditioning of tree seedlings on establisment and subsequent growth is inadequate. Therefore,the present study was carried out on *Robinia pseudoacacia* to test the hypothesis that conditioning treatments with placlobutrazol, moisture stress or their combination enable *Robinia* seedlings to better withstand subsequent conditions of water stress. *Robinia pseudoacacia* Linn (black locust) is a fast-growing nitrogen fixing multipurpose tree species, widely planted for soil amelioration, wasteland rehabilitation and control of soil erosion, besides being a good source of leaf fodder and fuelwood.

Materials and methods

Plant material

Robinia pseudoacacia seeds were germinated in polythene envelopes. Healthy and uniform 6-month-old seedlings were transplanted into plastic pots (7.5 litre capacity) filled with silty loam and farmyard manure (FYM) in 3:1 ratio. Plastic pots with a single seedling each were kept in the open and seedlings were allowed to grow at near field capacity for one more month with day temperature 26–31 °C and night temperature 16–20 °C.

Conditioning treatments

Conditioning treatments comprised moisture stress, paclobutrazol and their combination. Pots containing 7-month-old uniform seedlings of *R. pseudoacacia* were selected and divided into 6 treatment groups, each consisting of 180 seedlings. The treatments were: T_1, control; T_2, PP_{333}, 5 $\mu g\ g^{-1}$; T_3, PP_{333} 10 $\mu g\ g^{-1}$; T_4, stress cycle(s) only; T_5, PP_{333} 5 $\mu g\ g^{-1}$ + stress cycle(s); T_6, PP_{333} 10 $\mu g\ g^{-1}$ + stress cycle (s). Stress cycle (1, 2 or 3) treatments were superimposed on seedlings in T_4, T_5 and T_6.

Paclobutrazol (250 g l^{-1}) at 5 and 10 $\mu g\ g^{-1}$ with 0.01% Tween 20 as a surfactant was given as single foliar spray to all the seedlings in groups 2, 3, 5 and 6 whereas the control (T_1) seedlings were sprayed with an equal amount of water, 8 days before the commencement of moisture stress treatments. Seedlings were sprayed till runoff with the test solution. Moisture stress conditioning was imposed by withholding watering. Seedlings were kept in the open after treatment. Control and paclobutrazol treated (T_2 and T_3) seedlings were kept near field capacity during the entire conditioning period. Sixty seedlings each from treatment groups T_4 to T_6 were subjected to three consecutive stress cycles, each consisting of 5 days of withholding water followed by 5 days of rehydration. Similarly, 60 seedlings each from groups T_4 to T_6 were given two stress cycles whereas the remaining 60 seedlings in each treatment group (4–6) were subjected to one stress cycle.

Drought stress

Eight-month-old unconditioned and conditioned seedlings were subjected to water stress by withholding watering up to 16 days on 30 May. At the beginning of the stress period, all the pots were saturated with equal amounts of water in the evening. Soil moisture content after 12 h of saturation was 26.22% which declined to 8.12% after 8 days of stress and 6.76% after 16 days of stress. Sampling for different attributes was done on the 8th and 16th days of water stress.

Leaf water potential

Water potential (Ψ) was determined by the pressure chamber method of Scholander *et al.* (1965). A terminal twing (15 cm) containing young leaflets was enclosed in the chamber and pressure applied. Four replications of each treatment combination were made and expressed as MPa.

Metabolic indicators

Proline was estimated by the method of Singh *et al.* (1972) using methanol, chloroform and water (12:5:1) as an extraction solvent. For amino acid determination the method of Lee and Takahashi (1996) was followed. To 1 ml of the above extract was added 1.9 ml of the reaction mixture (1% ninhydrin solution in 0.5 M citrate buffer, 1.2 ml of 55% glycerol and 0.2 ml of 0.5 M citrate buffer). Sugars were estimated by the anthrone reagent method of Nelson (1944).

Drought injury index

Injury index *(Id)* was estimated by the method of Gebre and Kuhns (1991). Electrolyte leakage of 10 leaf discs of equal size was determined using a conductivity meter.

Statistical analysis

Data were statistically tested using analysis of variance in completely randomised block design with four replications.

Results

Shoot and root dry weights

The results indicate much greater reduction in shoot dry weight (71.2%) in unconditioned seedlings after 16 days of stress as compared to PP_{333} and PP_{333} + stress conditioned seedlings (Table 1). Comparison of treatment effect during cycle I (10 days), II (20 days), and III (30 days) at 8 days of stress show that T_1 (control) statistically (p=0.05) differs from treated plants T_2, T_3, T_5 and T_6 during all the three

Table 1. Shoot and root dry weights(g) in *Robinia pseudoacacia* when conditioned and unconditioned seedlings from cycle I (10 days), cycle II (20 days) and cycle III (30 days) were subjected to water stress up to 16 days. Moisture stress conditioned plants at 10 days had received 1 stress cycle, at 20 days 2 stress cycles and at 30 days 3 stress cycles.

Treatment	8 days stress			16 days stress		
	10 days	20 days	30 days	10 days	20 days	30 days
			Shoot			
Unconditioned (T_1)	12.6 (63.3)	17.0 (59.6)	17.0 (63.6)	11.0 (71.2)	12.6 (72.6)	12.0 (76.3)
PP_{333} 5 $\mu g\ g^{-1}$ (T_2)	25.6 (25.6)	28.0 (33.5)	27.2 (41.8)	24.6 (35.6)	22.6 (50.8)	21.6 (57.4)
PP_{333} 10 $\mu g\ g^{-1}$ (T_3)	24.0 (30.2)	30.6 (27.3)	30.4 (35.0)	25.6 (32.9)	24.6 (46.5)	21.4 (57.8)
Stress cycle(s) (T_4)	14.3 (58.1)	12.6 (70.1)	13.1 (72.0)	10.6 (72.2)	12.3 (73.6)	12.0 (76.3)
PP_{333} 5 $\mu g\ g^{-1}$ + stress cycle(s) (T_5)	22.6 (34.2)	20.6 (51.1)	19.4 (58.5)	23.3 (39.0)	23.3 (49.3)	21.2 58.2)
PP_{333} 10 $\mu g\ g^{-1}$ + stress cycle(s) (T_6)	24.6 (28.5)	26.6 (36.8)	25.7 (45.0)	19.0 (50.2)	21.6 (53.0)	17.4 (65.7)
LSD (0.05)	5.5	6.0	5.7	4.7	8.8	6.1
			Root			
Unconditioned	11.6 (31.7)	11.3 (52.5)	10.0 (58.9)	11.3 (44.6)	10.3 (62.6)	9.3 (66.5)
PP_{333} 5 $\mu g\ g^{-1}$	21.0 (+23.5)	18.6 (21.8)	18.1 (25.5)	18.3 (10.2)	22.0 (20.2)	21.4 (23.0)
PP_{333} 10 $\mu g\ g^{-1}$	21.3 (+25.2)	20.0 (15.9)	20.6 (15.2)	19.3 (5.3)	25.5 (9.4)	23.4 (15.8)
Stress cycle	6.0 (64.7)	6.0 (74.7)	5.4 (77.7)	5.0 (75.4)	9.0 (67.3)	8.6 (69.0)
PP_{333} 5 $\mu g\ g^{-1}$ + stress cycle	19.3 (+13.5)	20.0 (15.9)	18.0 (25.9)	21.0 (+2.9)	20.0 (27.5)	18.7 (32.2)
PP_{333} 10 $\mu g\ g^{-1}$ + stress cycle	17.0 (00.0)	15.3 (35.7)	14.1 (41.9)	16.3 (20.2)	18.6 (32.6)	16.1 (40.2)
LSD (0.05)	6.9	3.9	4.1	4.0	4.5	4.2

Parentheses indicate percentage inhibition relative to unconditioned daily watered control.

cycles (Table 1). The same was true for the 16 days of stress. Root dry weight inhibition in unconditioned seedlings after 16 days of stress ranged 45–67%. This was significantly greater than that in PP_{333} alone and PP_{333} + stress conditioned seedlings especially in cycles I and II (Table 1).

Water potential

The effect of conditioning cycle on water potential was more evident at 8 days stress (Figure 1a). PP_{333} + 1 stress cycle seedlings exhibited a higher water potential (ranging between - 0.376 and - 0.593 MPa) than the unconditioned seedlings (ψ = - 0.770 MPa). Moisture stress conditioned seedlings recorded lower water potential than PP_{333} alone or PP_{333} + stress conditioned seedlings (Figure 1a). Water potential further decreased in unconditioned seedlings after 16 days of stress where values ranged between - 1.340 and - 1.520 MPa for all the cycles (Figure 1b). In PP_{333}, PP_{333} + stress cycle conditioned and moisture stress conditioned seedlings, water potential was significantly higher than that in unconditioned seedlings after 16 days of stress (Figure 1b).

Proline

There was 77% increase in proline in unconditioned seedlings (df. control) when seedlings from cycle II were subjected to water stress up to 8 days (Table 2); however, the percentage increase in PP_{333}, PP_{333} + stress cycle and moisture stress conditioned seedlings was much higher than in unconditioned seedlings. Accumulation of proline was seen up to 16 days of stress, although absolute amounts were higher at 8 days of stress (Table 2). LSD (0.05) for treatment effect on proline accumulation between unconditioned and conditioned seedlings both at 8 as well as 16 days of stress exhibit statistical differences.

Amino acids and sugars

Unconditioned and conditioned seedlings during stress registered increased levels of amino acids, with the increase being significantly higher in PP_{333} conditioned seedlings (Table 3). PP_{333} 5 $\mu g\ g^{-1}$ and moisture stress conditioned seedlings after 8 days of water deficit registered 427% increase over the control. A similar trend was observed for seedlings in cycles II and III. Although amino acid accumulation was also observed after 16 days of stress, it was of lower magnitude compared to that at 8 days of stress (Table 3). Treatment effect on amino acid was statistically significant ($p = 0.05$) for both stress periods.

Statistically significant differences were observed in sugar content between unconditioned and conditioned seedlings for up to 16 days of water stress. Percentage accumulation of soluble sugar was much greater in conditioned seedlings at 8 days than at 16 days of water stress (Table 4). Unconditioned seedlings from all the three cycles recorded significant inhibition (31.7–40.4%) over the control after 16 days of stress, although conditioned seedlings were found to accumulate significant amounts of soluble sugars (Table 4) during the same period.

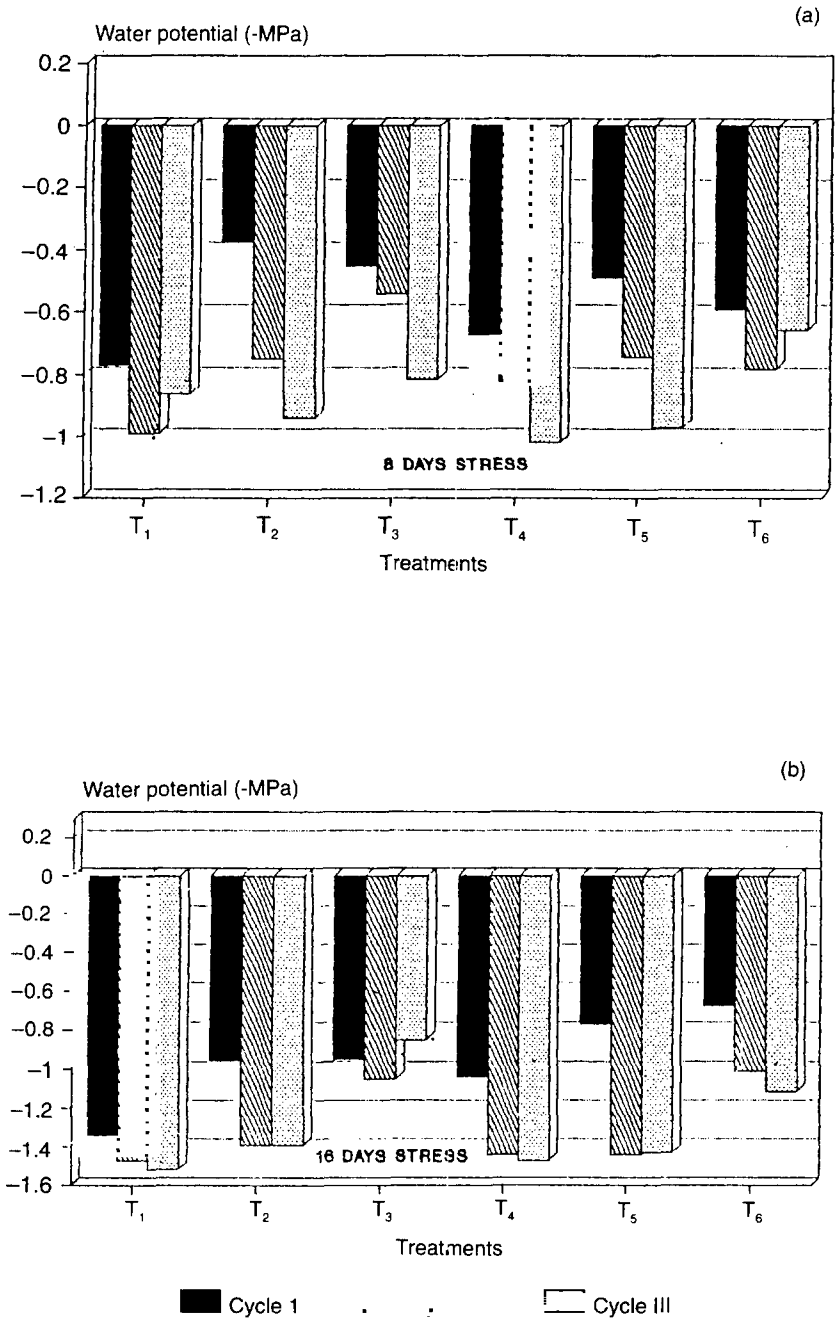

Figure 1. Leaf water potential (-MPa) in unconditioned and conditioned *Robinia pseudoacacia* seedlings during water stress up to 16 days. T_1 — unconditioned, T_2 — PP_{333} 5 µg g^{-1}, T_3 — PP_{333} 10 µg g^{-1}, T_4 — stress cycle, T_5 — PP_{333} 5 µg g^{-1} + stress cycle, T_6 — PP_{333} 10 µg g^{-1} + stress cycle. CD (0.05), level of significance to compare T_1 to T_6 on 8th day of stress: under cycle I (10 days) — 0.107, cycle II (20 days) — 0.217, cycle III (30 days) — 0.202. CD (0.05) on 16th day of stress: under cycle I (10 days) — 0.275, cycle II (20 days) — 0.199, cycle III (30 days) — 0.120.

Table 2. Proline content (µg g^{-1} fresh wt.) in *R. pseudoacacia* when seedlings from cycle I (10 days), cycle II (20 days) and cycle III (30 days) were subjected to water stress up to 16 days. Moisture stress conditioned plants at 10 days had received 1 stress cycle, at 20 days 2 stress cycles and at 30 days 3 stress cycles.

Treatment	8 days stress			16 days stress		
	10 days	20 days	30 days	10 days	20 days	30 days
Unconditioned (T_1)	146.2 (76.1)	176.0 (77.0)	166.0 (45.20)	199.2 (42.2)	197.1 (40.7)	160.4 (6.90)
PP_{333} 5 µg g^{-1} (T_2)	196.0 (136.0)	241.0 (142.3)	265.0 (131.8)	313.0 (123.5)	309.3 (120.9)	286.4 (90.0)
PP_{333} 10 µg g^{-1} (T_3)	214.0 (157.8)	287.5 (189.0)	297.4 (160.1)	346.0 (147.1)	336.0 (140.0)	261.4 (74.2)
Stress cycle(s) (T_4)	163.0 (96.3)	212.0 (113.00)	227.4 (98.9)	267.0 (90.7)	233.1 (66.4)	186.1 (74.0)
PP_{333} 5 µg g^{-1} + stress cycle(s) (T_5)	246.4 (196.8)	286.9 (188.5)	297.0 (160.0)	331.5 (136.7)	304.0 (117.1)	276.0 (84.0)
PP_{333} 10 µg g^{-1} + stress cycle(s) (T_6)	194.0 (137.7)	249.0 (150.4)	262.4 (129.6)	304.0 (116.4)	286.5 (104.6)	224.0 (49.3)
LSD (0.05)	39.9	51.2	15.3	8.7	11.8	24.6

Parentheses indicate percentage values relative to untreated daily watered control.

Table 3. Amino acid (mg g^{-1} dry wt.) in *R. pseudoacacia* when conditioned and unconditioned seedlings from cycle I (10 days), cycle II (20 days) and cycle III (30 days) were subjected to water stress up to16 days. Moisture stress conditioned plants at 10 days had received 1 stress cycle, at 20 days 2 stress cycles and at 30 days 3 stress cycles.

Treatment	8 days stress			16 days stress		
	10 days	20 days	30 days	10 days	20 days	30 days
Unconditioned (T_1)	15.3 (83.3)	20.8 (167.0)	21.9 (134.9)	18.1 (76.4)	19.9 (109.0)	19.1 (70.0)
PP_{333} 5 µg g^{-1} (T_2)	34.9 (318.0)	33.0 (324.0)	30.2 (224.0)	32.3 (216.0)	32.2 (239.1)	29.1 (159.3)
PP_{333} 10 µg g^{-1} (T_3)	31.2 (247.1)	28.9 (271.8)	29.1 (213.0)	27.8 172.0)	25.9 (172.8)	23.2 (107.1)
Stress cycle(s) (T_4)	29.8 (257.7)	27.3 (250.8)	28.2 (203.3)	27.6 (170.0)	21.3 (123.0)	20.2 (80.3)
PP_{333} 5 µg g^{-1} + stress cycle(s) (T_5)	44.0 (427.0)	38.6 (396.7)	36.9 (296.2)	36.7 (290.0)	27.0 (183.6)	23.3 (107.5)
PP_{333} 10 µg g^{-1} + stress cycle(s) (T_6)	40.2 (382.0)	41.6 (435.3)	39.6 (325.8)	37.2 (263.9)	28.5 (200.0)	27.8 (147.7)
LSD (0.05)	3.32	1.47	2.48	4.39	2.11	1.77

Parentheses indicate percentage inhibition relative to untreated control.

Table 4. Soluble sugar changes (mg g^{-1} dry wt.) in *R. pseudoacacia* when conditioned and unconditioned seedlings from cycle I (10 days), cycle II (20 days) and cycle III (30 days) were subjected to water stress up to 16 days. Moisture stress conditioned plants at 10 days had received 1 stress cycle, at 20 days 2 stress cycles and at 30 days 3 stress cycles.

Treatment	8 days stress			16 days stress		
	10 days	20 days	30 days	10 days	20 days	30 days
Unconditioned (T_1)	10.1 (19.1)	12.5 (24.3)	12.1 (6.0)	8.1 (-31.7)	9.0 (-33.7)	8.5 (-40.4)
PP_{333} 5 µg g^{-1} (T_2)	17.7 (108.9)	18.2 (81.8)	16.6 (44.9)	21.2 (78.7)	21.6 (60.6)	20.4 (43.0)
PP_{333} 10 µg g^{-1} (T_3)	14.8 (75.4)	14.6 (45.8)	15.5 (35.2)	17.2 (45.3)	19.9 (29.2)	18.2 (1.1)
Stress cycle(s) (T_4)	23.4 (176.5)	24.5 (144.7)	22.3 (94.8)	18.2 (53.1)	17.4 (29.2)	14.4 (1.1)
PP_{333} 5 µg g^{-1} + stress cycle(s) (T_5)	27.5 (22.5)	27.9 (178.2)	24.2 111.9)	24.0 (102.3)	19.2 (42.2)	18.6 (32.0)
PP_{333} 10 µg g^{-1} + stress cycle(s) (T_6)	24.7 (191.7)	22.0 (199.2)	23.0 (100.2)	19.3 (62.9)	18.2 (35.2)	17.3 (30.2)
LSD (0.05)	2.7	1.92	1.71	1.38	1.85	2.44

Parentheses indicate relative percentage value over the control.

Drought injury index

Irrespective of stress cycles the injury index in PP_{333} + moisture stress conditioning seedlings was significantly lower compared to unconditioned seedlings (Figure 2). Injury index in unconditioned *Robinia* seedlings on the 16th day of stress period averaged 69.9%, whereas the same for preconditioning treatments was much less and varied between 23 and 36% (Figure 2).

Discussion

Paclobutrazol and moisture stress conditioning in the present study were found to influence the physiological status which may help the plants cope with water stress conditions. Different conditioning treatments enhanced drought adaptation and minimised the deleterious effects of subsequent water deficit by i) checking growth, ii) controlling water potential, iii) changing the pace of metabolic activities, or iv) combination of all. Shoot and root dry weight reduction was significantly less in conditioned seedlings as compared to unconditioned seedlings during subsequent water stress. It is interesting that the pazlobutrazol treatments were more effective in curtailing dry weight loss than moisture stress conditioning up to 16 days of stress. This is important because it reflects the improved water relations in conditioned seedlings.

Leaf water potential is a major indicator of drought status in plants. During this study P_{333} alone and in combination with moisture stress cycles enabled *R. pseudoacacia* seedlings to maintain a relatively higher internal water content. This is substantiated by the findings that the injury index in conditioned seedlings under subsequent water

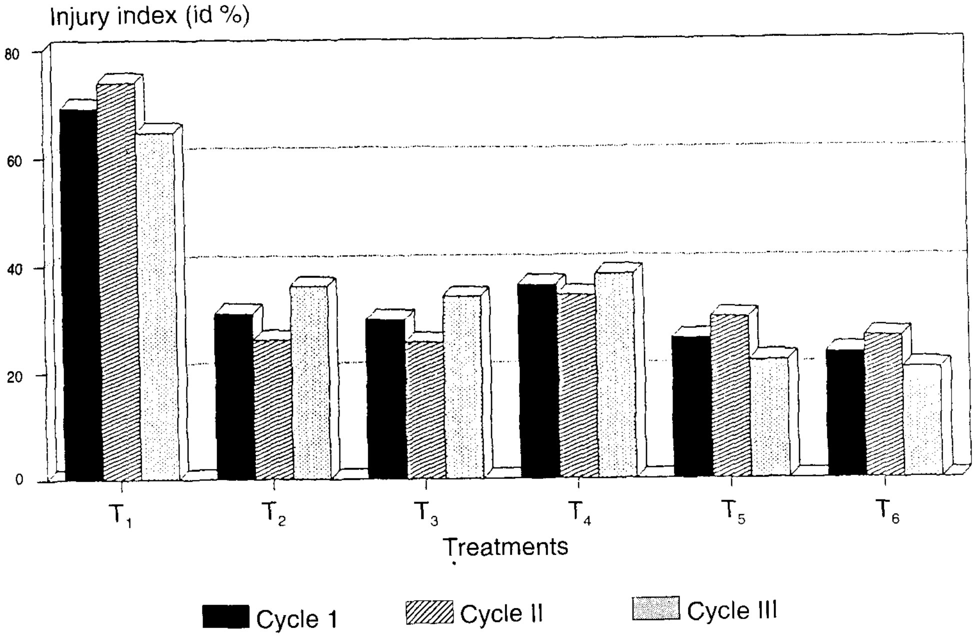

Figure 2. Injury index (*Id*) in unconditioned and conditioned *R. pseudoacacia* seedlings on 16th day of stress. T_1 — unconditioned, T_2 — PP_{333} 5 $\mu g\ g^{-1}$, T_3 — PP_{333} 10 $\mu g\ g^{-1}$, T_4 — stress cycle, T_5 — PP_{333} 5 $\mu g\ g^{-1}$ + stress cycle, T_6 — PP_{333} 10 $\mu g\ g^{-1}$ + stress cycle. LSD (0.05), level of significance to compare different treatments: under cycle I (10 days) – 5.00, cycle II (20 days) — 7.86, cycle III (30 days) — 6.98.

deficits was significantly less than in unconditioned seedlings. Further, there was statistically significant ($p = 0.01$) interaction where higher hydration of protoplasm (higher ψ) in conditioned seedlings was negatively correlated with injury index. Higher water potential in seedlings will maintain metabolic activities resulting in maintaining higher turgidity through osmoregulation. Moreover, the greater responsiveness and adaptability of conditioned seedlings during the drought phase are mainly amenable for maintaining higher concentrations of amino acid and sugars. It seems that active osmoregulation and turgor maintenance do not occur prior to drought in the conditioned plants, but conditioned plants are able to conserve water (i.e. higher water potential) during the onset of drought, allowing metabolic adjustment. This is a desirable character. The involvement of osmolytes in osmotic adjustment is known (Jones *et al.* 1980, Morgan 1984). Zwiazek and Blake (1989) and Thakur (1994) have also observed higher turgor in conditioned plants.

In the present study conditioning treatments in the order, paclobutrazol 10 $\mu g\ g^{-1}$ + one stress cycle > paclobutrazol 5 $\mu g\ g^{-1}$ + one stress cycle > one stress cycle only, seem to enable seedlings to better withstand subsequent drought by decreasing injury index and maintaining higher water potential. So, it is suggested that paclobutrazol at 10 $\mu g\ g^{-1}$ in combination with one stress cycle be adopted to condition nursery stock of *R. pseudoacacia* for better establishment and growth at dry sites.

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