Results

RESULTS

Callus Initiation and Growth

Inoculation of dehusked seeds of both rice varieties on to L.S. medium supplemented with 11.3 μM of 2,4-D led to callus initiation on day 4. It has been observed that the callus is derived from the scutellum (Plate 1a and b). The growth of callus followed a typical pattern registering the maximum growth by the end of fourth week. Irrespective of various treatments, the growth pattern remained almost same. The calli under nonstressed condition were friable and whitish in colour while those grown on hydroxyproline were slightly compact and straw coloured (Plate 2a and b).

Incorporation of NaCl at 200 mM concentration in the medium resulted in a significant reduction in the dry weight of calli of both cultivars (Fig.1, Table 3).A reduction of 50% and 42% was observed in the dry weights of calli of GR_{11} and BR respectively at the end of fourth week. However, hydroxyproline exposed cells when cultured on saline medium showed better growth compared to the salt control (Plate 3a and b). Addition of hydroxyproline into the saline medium improved further the growth of hydroxyproline exposed cells by 33% and 40% respectively in BR and GR_{11} (Fig. 1).

Na^+, K^+, Cl^-, Mg^{2+} and Ca^{+2} contents

Figure 2 shows the content of Na^+ in callus cultures grown under different treatments. During various periods of growth, there was not much difference between the sodium contents of the calli of both rice cultivars. Also, it is

Plate 1

a) Origin of callus (c) from scutellum (arrow) of BR variety x 40

b) Origin of callus (c) from scutellum (arrow) of GR_{11} variety x 40

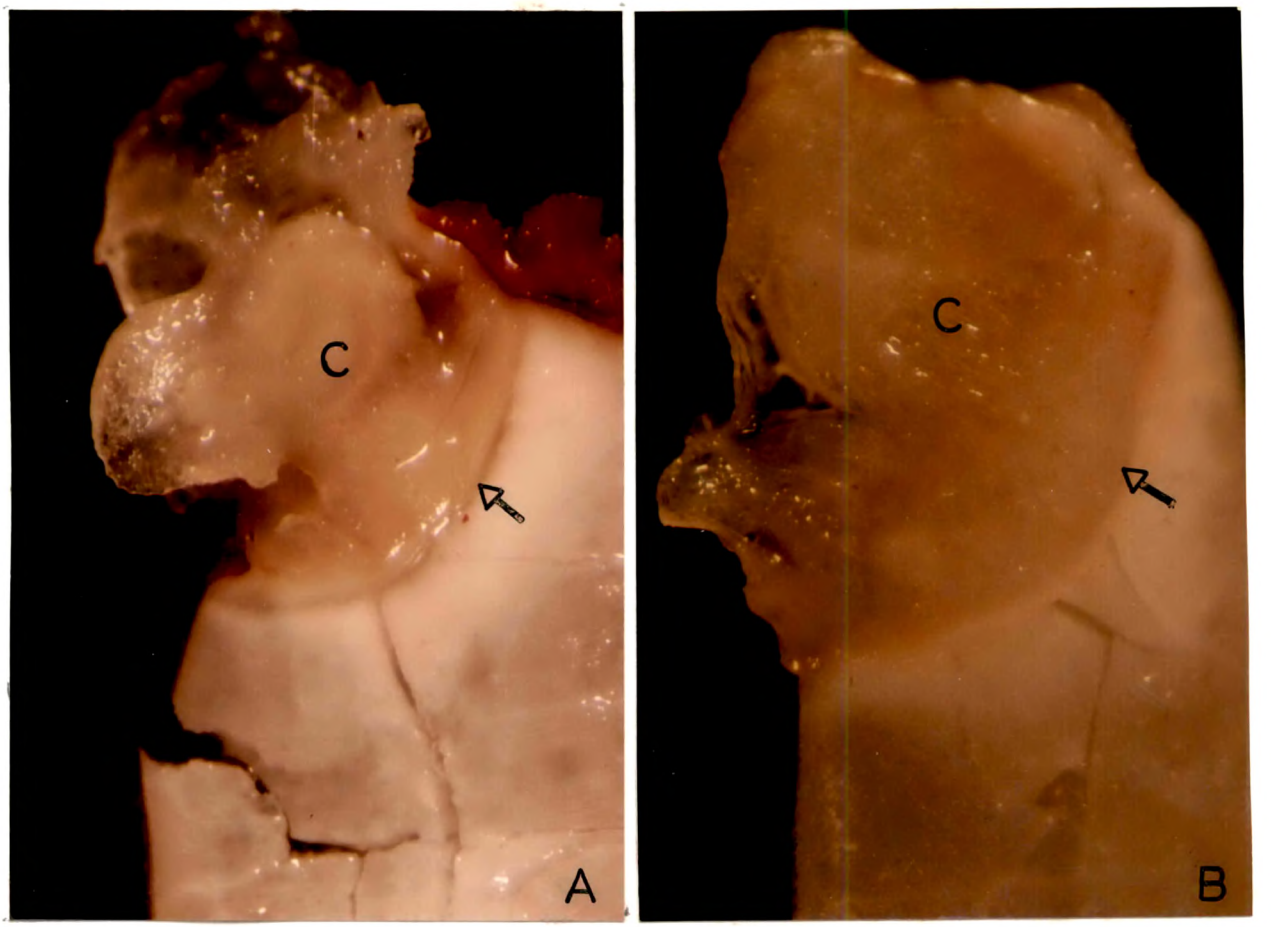

C
A
C
B

Plate 2

a) Four week old callus of BR variety on L.S. medium containing 0 (BRC) and 10 mM (BRT) concentration of hydroxyproline

b) Four week old callus of GR_{11} variety on L.S. medium containing 0 (GRC) and 10 mM (GRT) concentration of hydroxyproline.

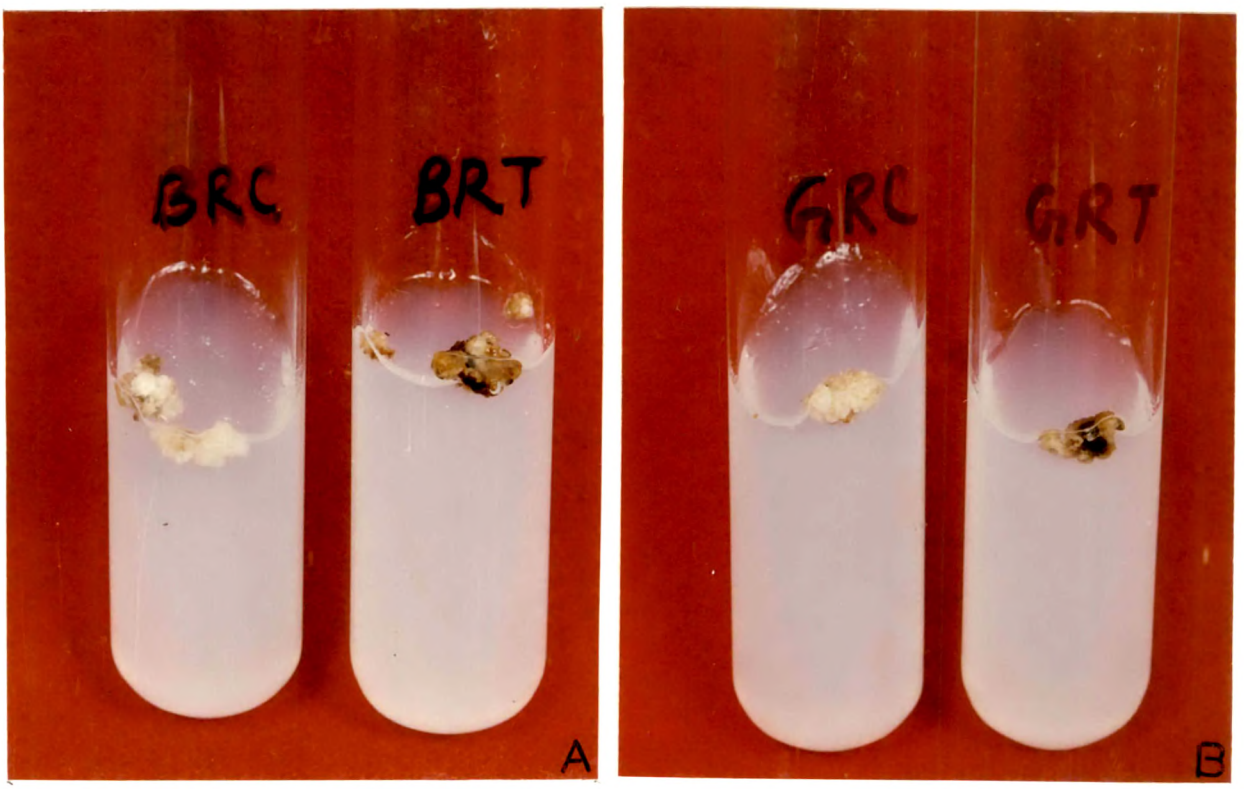

BRC
BRT
A
GRC
GRT
B

Plate 3 a) Four week old callus of BR variety under different treatments

BRC (L.S.medium)

BRT (L.S.medium + 10 mM hydroxyproline)

BRC (S) (L.S.medium + 200 mM NaCl)

BRT (S) (Hydroxyproline treated callus on L.S. medium + 200 mM NaCl)

BRT (S+H) (Hydroxyproline treated callus on L.S.medium + 200 mM NaCl + 10 mM hydroxyproline)

b) Four week old callus of GR variety under different treatments

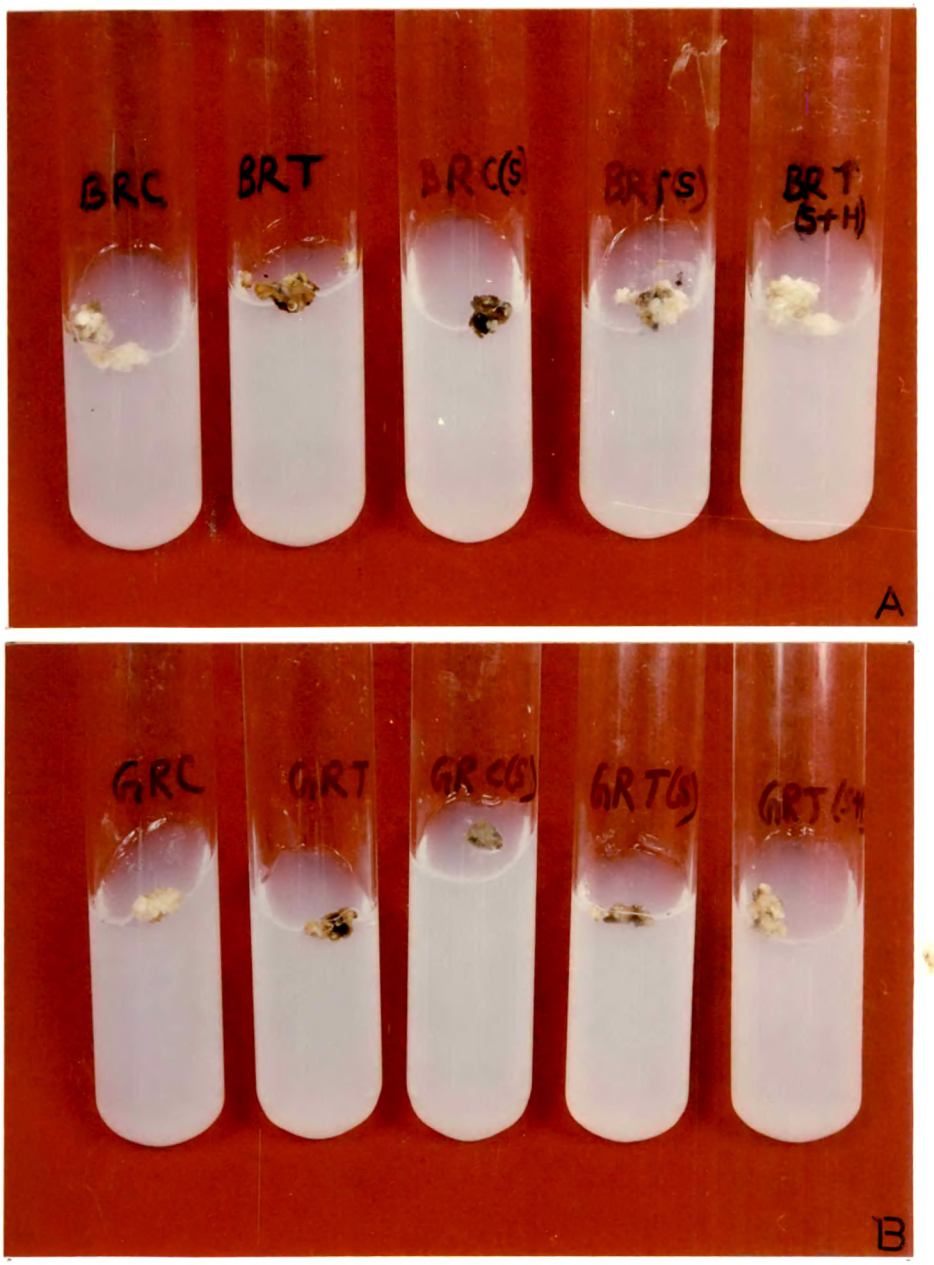
GRC (L.S.medium)

GRT (L.S.medium + 10 mM hydroxyproline)

GRC (S) (L.S.medium + 200 mM NaCl)

GRT (S) (Hydroxyproline treated callus on L.S. medium + 200 mM NaCl)

GRT (S+H) (Hydroxyproline treated callus on L.S.medium + 200 mM NaCl + 10 mM hydroxyproline)


BRC
BRT
BRC(S)
BRT(S)
BRT (S+H)
A
GRC
GRT
GRC(S)
GRT(S)
GRT(S+H)
B

observed that incorporation of hydroxyproline into the medium did not bring about any significant change in the sodium content of the cells, as compared to the control (Table 4). The maximum accumulation of sodium was discerned at the end of fourth week in calli exposed to 200 mM NaCl. A 20 and 23 fold accumulation of Na^+ was observed in BR and GR_{11} cultivar tissues respectively, compared to the control. However, a significant reduction was observed in the Na^+ content of hydroxyproline pre-exposed calli growing on NaCl containing medium.

Hydroxyproline exposed BR and GR calli accumulated 20% and 25% less Na^+ respectively, as compared to their salt controls, when grown on L.S. medium containing both hydroxyproline and NaCl (Fig. 2).

The K^+ content of the calli grown under various treatments is shown in the Figure 3. Control BR callus accumulated nearly three fold more K^+ at the end of the fourth week as compared to 0 day, while callus of GR_{11} showed 2.7 fold more accumulation of K^+. In response to salinization, both rice cultivars registered a fall in their K^+ content (Table 5). At the end of fourth week, salinized BR and GR_{11} calli exhibited 1.4 fold and 1.9 fold respectively less K^+ than that of their controls.

Prior exposure to hydroxyproline helped the cells in maintaining comparatively higher level of K^+. Calli of both rice cultivars growing on medium containing both hydroxyproline and NaCl maintained nearly the same level of K^+ as that of

their control (i.e. there was an increase of 38% in BR cultivar and 33% in GR_{11} cultivar over their salt control values).

There was not much of difference in Cl^- content of the calli of the two rice cultivars. However, when the calli were grown on NaCl containing medium, there was a significant accumulation of Cl^- ions in both the cultivars (Table 6). As compared to the pure control, BR and GR_{11} calli showed a 4 and 5 fold increase respectively in their levels of Cl^- ions at the end of fourth week. In this case also hydroxyproline helped the cells in reducing their Cl^- content significantly. Hydroxyproline treated BR salinized callus growing on medium containing both NaCl and hydroxyproline accumulated 16% less Cl^- as compared to its salt control while GR_{11} salinized callus showed 18% less accumulation of Cl^- (Fig. 4).

The Mg^{2+} content of the calli was highest during the period of maximum growth (Fig.5). It is observed that under all treatments the level of Mg^{2+} was higher in the tolerant BR callus culture as compared to the susceptible GR culture.

Upon salinization, a 1.9 fold and 1.4 fold increase in the content of Mg^{2+} over the control was discerned in the callus cultures of BR and GR_{11} respectively. There was further increase in the Mg^{2+} level of both the rice cultivars which had been exposed to hydroxyproline and then grown on NaCl and hydroxyproline containing medium (Table 7).

Figure 6 shows the pattern of Ca^{2+} accumulation under various treatments. The level of Ca^{2+} in the callus cultures rose progressively parallel to growth and

reached the maximum value at the end of fourth week. Like the Mg^{2+} content, the Ca^{2+} calcium content of the susceptible GR_{11} cultivar was comparatively lower at all times than that of the tolerant BR cultivar. BR control callus accumulated three times more Ca^{2+} while GR_{11} callus showed nearly two fold rise in Ca^{2+} as compared to 0 day, at the end of fourth week. Administration of NaCl into the medium enhanced the accumulation of calcium ions in both the cultivars (Fig. 6, Table 8). As compared to their controls, BR salinized callus showed nearly 1.9 fold and GR_{11} salinized callus exhibited 1.8 fold increase in its content of calcium ions.

Maximum content of Ca^{2+} was observed in the calli growing on NaCl and hydroxyproline containing medium. Pre-exposure of the cells to hydroproline helped them in maintaining high levels of Ca^{2+} (BR callus exhibited 2.9 fold and GR_{11} callus exhibited 2.7 fold increase over the pure control).

Free Proline Content

The free proline content of both rice cultivars rose progressively from the second week, reaching the maximum level at the end of sixth week (Fig. 7). This pattern of proline accumulation was common to all calli grown under various treatments. It is interesting to note that there was significant increase after two weeks in the proline level, while after four weeks, comparatively, the increase was not that pronounced.

The free proline content of control calli of both rice cultivars was very low prior to the imposition of either hydroxyproline or salt treatment. BR callus when

grown on hydroxyproline containing medium registered a 3 fold increase in its proline content as compared to the control, while GR_{11} callus showed nearly 2 fold increase over its control at the end of sixth week. Salinization of callus also resulted in a 2 fold and 3.4 fold increase in proline levels of BR and GR_{11} respectively as compared to their controls (Fig.7), at the end of the sixth week.

The most significant changes in proline concentration took place at the end of sixth week in hydroxyproline exposed calli grown on medium containing both hydroxyproline and NaCl (Table 9). The proline level increased to a maximum 5 times that of control in BR callus, accounting for a maximum 4.3% of the callus fresh weight (Fig. 7). In GR_{11} callus, as compared to control, the level of proline was nearly six times more, accounting for a maximum of 2.7% callus fresh weight (Fig. 7).

Proline Oxidase Activity

The calli of both rice varieties showed a decrease in their activity of proline oxidase from second week onwards (Fig. 8). The activity of proline oxidase was comparatively higher in the non-stressed calli of both rice varieties. However, incorporation of NaCl in the medium led to significant decrease in the proline oxidase activity (at the end of the sixth week, Table 10). The stressed BR callus showed nearly 41% decrease while GR_{11} stressed callus showed a 15% decrease in its proline oxidase activity as compared to the control (Fig. 8). A further decrease in the activity of the proline oxidase was discerned in calli which were pre-exposed to hydroxyproline and then grown on medium containing both NaCl and hydroxyproline. In this case, BR callus exhibited nearly 56% reduction in

its proline oxidase activity (as compared to control) while GR_{11} callus registered almost 34% reduction.

Total Protein Content

The calli of both rice cultivars under all treatments exhibited a steady increase in their protein contents from second week and reached the maximum level at the end of the sixth week. The control callus of BR, as compared to 0 day, registered nearly 1.7 fold increase in its protein content, while GR_{11} control callus showed nearly 3.7 fold increase at the end of the sixth week (Fig. 9). A pronounced stimulation of protein content was visible when the cells were grown on medium containing hydroxyproline. As compared to 0 day, a 6.6 fold and 7.1 fold increase was found in the protein level of BR and GR_{11} callus respectively, at the end of the sixth week. At this time, in BR the protein content accounted for 3.14% callus fresh weight and in GR_{11} it accounted for 3.18% callus fresh weight.

NaCl caused a significant reduction in the protein level of the callus cultures of both rice varieties (Table 11). In BR salinized callus, there was 35% reduction in the protein content as compared to the control (Fig. 9) while in salinized GR_{11} callus, 75% reduction was observed at the end of the sixth week. However, significant stimulation was observed in the protein level of the cells which were pre-exposed to hydroxyproline and then grown on saline medium. In this case, as compared to their salt controls, the hydroxyproline exposed BR callus growing on NaCl medium containing hydroxyproline showed 45% increase in its protein content while GR_{11} callus showed nearly 58% increase (at the end of the sixth week).

The level of polyamines from second week onwards rose parallel to growth, reaching the maximum at the end of fourth week. However, from fourth to sixth week, the polyamine content of tissues did not show any significant change. Incorporation of hydroxyproline in the medium did not affect the level of putrescine markedly (at the end of fourth week, Table 12). However, under NaCl stress, the level of putrescine showed an increase of nearly 24% in BR callus, while GR_{11} callus registered an increase of nearly 30% (Fig. 10), as compared to its control (at the end of fourth week). It is interesting to note that under stress conditions, hydroxyproline reduced the content of putrescine in both rice varieties. As compared to its salt control, hydroxyproline pretreated BR callus growing on medium containing both NaCl and hydroxyproline showed 11% reduction while GR_{11} callus exhibited 18% reduction in its putrescine content (at the end of the fourth week).

Unlike putrescine, the level of spermine was greatly enhanced by hydroxyproline (Table 13). It was observed that BR callus growing on hydroxyproline containing medium showed 85% increase, while GR_{11} callus showed a 41% increase in its spermine content during the period of maximum growth (Fig. 11). NaCl reduced the spermine content in both varieties. After four weeks of growth on salt containing medium, BR callus showed 10% decline in its spermine content (Fig. 11), while GR_{11} callus showed a decline of 42%. A noticeable enhancement in the spermine level of salinized calli was brought about by hydroxyproline (Table 13). Hydroxyproline pretreated tolerant and susceptible calli growing on medium containing both NaCl and hydroxyproline

showed 98% and 56% increase respectively in spermine level (as compared to the corresponding salt control).

As compared to spermine, spermidine level increased only slightly in BR (26% more than control) and GR_{11} (10%) callus growing on hydroxyproline containing medium at the end of fourth week. Incorportion of NaCl in the medium reduced the level of spermidine in tissues. At the end of fourth week, calli of BR and GR_{11} varieties under the influence of NaCl showed 18% and 46% reduction respectively in their spermidine contents (Fig.12)

However addition of hydroxyproline into the medium partially reversed the salt induced inhibition of spermidine level in the salinized calli. As compared to salt control, BR callus (pretreated with hydroxyproline) growing on medium containing both NaCl and hydroxyproline exhibited a significant increase of 58% while GR_{11} callus showed 48% increase in its level of spermidine (Table 14).

IAA Oxidase Activity

The IAA oxidase activity of the calli of both rice cultivars followed a different pattern as compared to the activity of other enzymes. The lowest activity of IAA oxidase was observed at the end of fourth week (period of maximum growth) in calli grown under different treatments. Salinization of the callus significantly enhanced the IAA oxidase activity in both cultivars (Table 15). At the end of fourth week, the enzyme activity of BR callus growing on NaCl containing medium was nearly 1.2 times more than its corresponding control value, while GR_{11} salinized callus exhibited nearly 1.9 times more enzyme activity (Fig. 13).

However, prior exposure to hydroxyproline helped the cells in maintaining a comparatively low level of IAA oxidase activity. Hydroxyproline exposed BR callus when grown on medium containing both NaCl and hydroxyproline showed nearly a significant reduction of 34% in the enzyme activity as compared to its salt control (Fig. 13), while GR_{11} callus exhibited 9% less activity as compared to its salt control, at the end of fourth week.

Total Amylase Activity

The amylase activity in the tissues was initially low and it rose progressively till the fourth week and declined thereafter to a low level in case of both the cultivars. It is interesting to note that a nearly 6 fold and 7 fold increase in the total amylase activity was observed at the end of the fourth week over the 0 day reading respectively in calli of BR and GR_{11} grown on hydroxyproline containing medium (Fig. 14).

NaCl was found to be highly inhibitory to amylase activity as the activity of the enzyme decreased significantly by 69% in BR and by 84% in GR_{11} salinized tissues as compared to the control (at the end of fourth week, Table 16). However, hydroxyproline exposure caused a significant stimulation of amylase activity in both cultivars. Hydroxyproline exposed BR callus when grown on medium containing both hydroxyproline and NaCl showed nearly 56% increase in amylase activity while GR_{11} callus showed an increase of 59% as compared to its salt control (Fig. 14).

Acid Invertase Activity

The activity of acid invertase was highest at the end of the fourth week in callus cultures of both rice cultivars grown under various treatments. Maximum invertase activity was exhibited by the cultures grown on medium containing hydroxyproline. At the end of fourth week, as compared to the control, the cells of both rice cultivars grown under the influence of hydroxyproline exhibited nearly 1.6 times more invertase activity (Fig. 15).

NaCl significantly reduced the activity of invertase enzyme (Table 17). At the end of fourth week BR salinized callus showed 43% reduction in its invertase activity while GR_{11} salinized callus exhibited a more severe reduction of 65% (Fig. 15). However, pre-treatment of the callus with hydroxyproline helped the cells in maintaining more activity of invertase. Hydroxyproline exposed BR callus showed a significant rise of 29% while GR_{11} callus showed 56% rise in their invertase activity (at the end of fourth week), when grown on medium containing both NaCl and hydroxyproline.

Cellulase Activity

The activity of cellulase in the control calli rose steadily till the end of fourth week after which it declined (Fig. 16). Maximum activity of cellulase was observed in calli grown on hydroxyproline containing medium. As compared to 0 day, BR callus growing on hydroxyproline containing L.S. medium showed nearly 3 fold increase in cellulase activity while GR_{11} callus showed nearly 1.5 times more activity, at the end of fourth week.

Plate 4 a) Callus of BR variety showing regeneration on MS medium

b) Callus of GR_{11} variety showing regeneration on MS medium

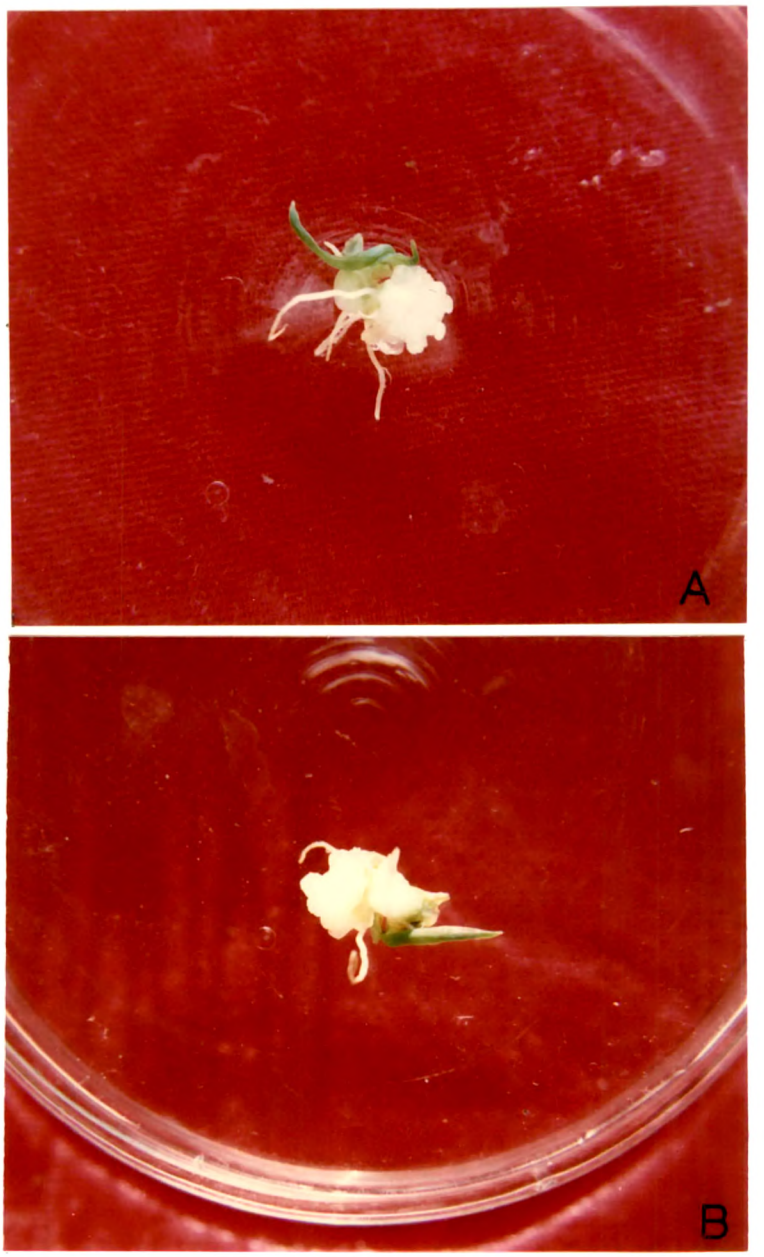
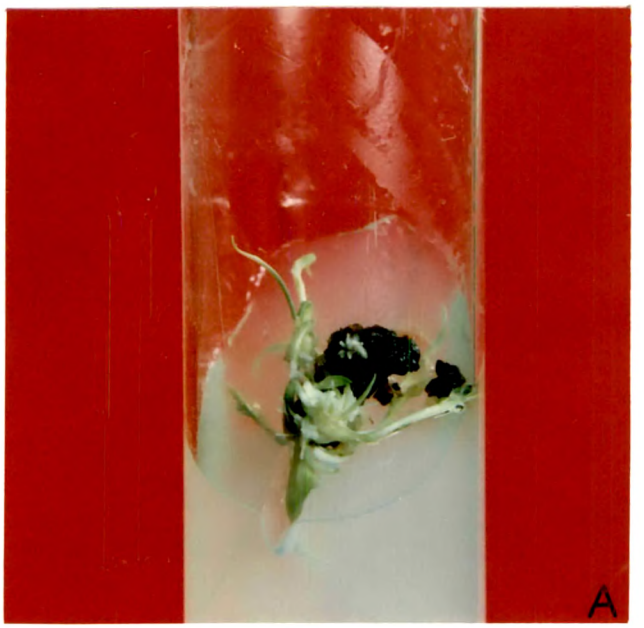

A
B

Plate 5 a) Hydroxyproline resistant callus of BR variety showing regeneration on MS medium

b) Hydroxyproline resistant callus of GR_{11} variety showing regeneration on MS medium


A


B

Plate 6

a) Callus of BR variety showing regeneration on MS medium containing 200 mM NaCl

b) Callus of GR_{11} variety showing regeneration on MS medium containing 200 mM NaCl


A


B

Plate 7

a) Hydroxyproline exposed callus of BR variety showing regeneration on MS medium containing 200 mM NaCl and 10 mM hydroxyproline

b) Hydroxyproline exposed callus of GR_{11} variety showing regeneration on MS medium containing 200 mM NaCl and 10 mM hydroxyproline


A


B

Plate 8 Growth of regenerated plantlets on MS medium containing 200 mM NaCl and 10 mM hydroxyproline.

The cellulase activity in the salt stressed calli also followed a similar trend as that of control but a significant reduction in the activity was observed in both rice cultivars (Table 18). In presence of NaCl, BR callus exhibited almost 59% reduction in its cellulase activity at the end of fourth week while GR_{11} callus showed 67% reduction.

It is interesting to note that when BR callus treated with hydroxyproline was grown on NaCl containing medium, it showed 45% more cellulase activity as compared to salt control, while GR_{11} callus showed an increase of 30%. This activity was further enhanced by incorporation of hydroxyproline in medium containing NaCl; in this case the activity was 63% and 70% more in BR and GR_{11} callus respectively (Fig.16).

Regeneration of Hydroxyproline Resistant Calli

Culturing of callus tissues of both rice varieties on MS medium supplemented with 3% sucrose, 13.32 μM BA and 2.46 μM IBA resulted in plantlet regeneration. Callus showed formation of healthy green shoots with good growth of roots (Plate 4a and b). Hydroxyproline resistant calli of both rice cultivars when grown on the same medium also showed plantlet regeneration (Plate5a and b). A poor regeneration was observed under NaCl stress. The calli growing on medium containing NaCl showed poor growth of the regenerants (Plate 6a and b), as compared to the control. However, hydroxyproline pre-exposed callus growing on medium containing both salt and hydroxyproline showed better regeneration with good growth of both shoots and roots (Plate 7a and b). Further growth of the plantlets occured when they were transferred to above fresh medium containing hydroxyproline and NaCl (Plate 8).

Table 3 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on growth (mg dry weight) of rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@19±0.4	38±0.6	72±0.6	70±1.4
HP	19±0.4	36±0.9*	41±0.9*	41±0.9*
NaCl	13±0.5	26±0.7*	42±0.9*	41±0.9*
HP exposed cells to NaCl	16±0.5	34±1.3•	50±0.9*•	49±1.0*•
HP exposed cells to NaCl + HP	16±0.4	38±0.7*•	56±1.3*•	54±1.3*•
(B)				
Control	20±0.9	40±1.0	60±1.1	60±3.5
HP	20±0.9	30±0.7*	32±0.6*	32±0.6*
NaCl	18±1.0	27±0.7*	30±1.0*	30±1.0*
HP exposed cells to NaCl	18±1.0	29±0.8*	39±1.3*•	38±1.6*•
HP exposed cells to NaCl+HP	18±0.9	29±0.7*	42±1.2*•	42±1.2*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig 1. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on growth of rice callus of BR and GR_{11} varieties.

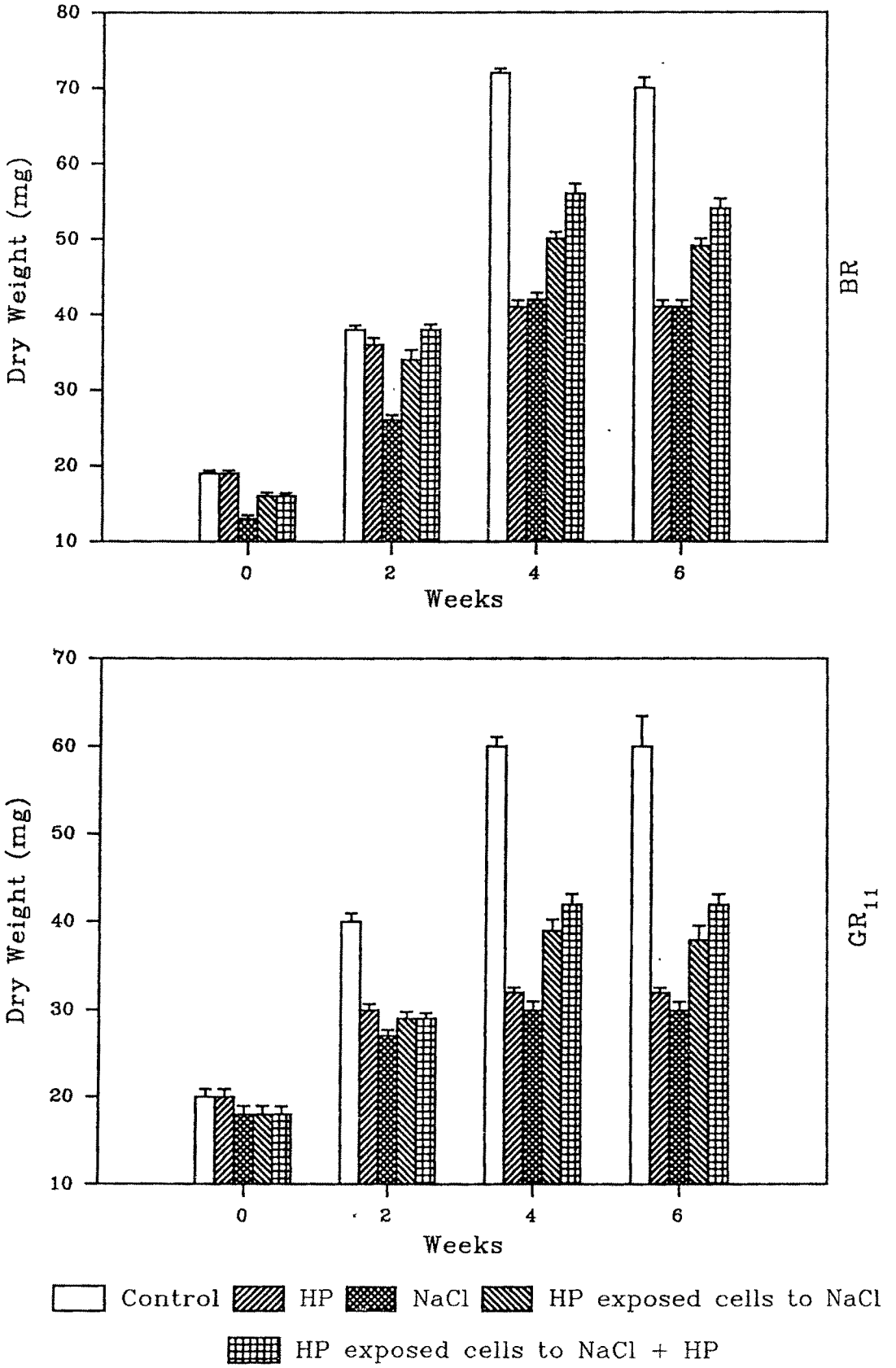


Table 4 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of Na^+ (mg g^{-1}dry wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@6.0±0.4	5.8±0.7	6±0.8	5.1±0.6
HP	6.0±0.4	6.2±0.3	6.5±0.4	5.3±0.6
NaCl	5.8±0.7	100±3.4*	120±2.9*	116.3±2.3*
HP exposed cells to NaCl	6.2±0.3	85.8±2.9*•	100±3.2*•	95±2.7*•
HP exposed cells to NaCl+HP	6.2±0.3	80.5±1.1*•	95.5±3.2*•	92±2.1*•
(B)				
Control	7.5±0.6	6.8±0.7	7.2±0.6	7±0.7
HP	7.5±0.6	7±0.6	7.4±0.7	7.1±0.6
NaCl	6.8±0.7	128±2.4*	165.7±3.2*	160.8±2.9*
HP exposed cells to NaCl	7±0.6	118.3±1.9*•	130±3.0*•	128±2.6*•
HP exposed cells to NaCl+HP	7±0.6	110.6±4.3*•	125.2±4.3*•	122±2.6*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 2. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on Na^+ content in rice callus of BR and GR_{11} varieties.

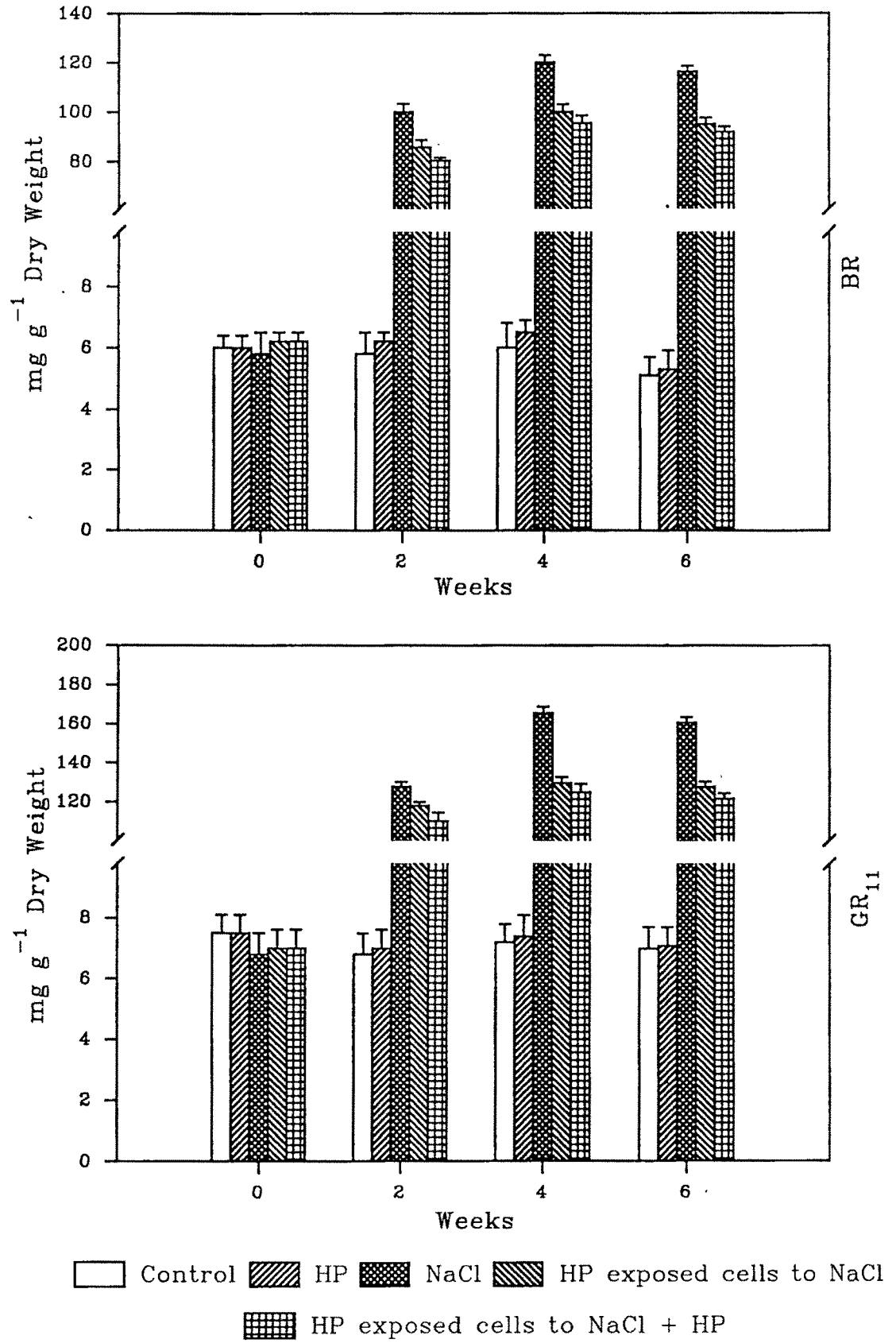


Table 5 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of K^+ (mg g^{-1}dry wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@6.2±0.4	16±1.3	18±1.0	17±0.7
HP	6.2±0.4	13±0.7*	17±0.7	16±0.7
NaCl	16*±1.3	12.5±0.7*	13±0.6*	12.5±0.6*
HP exposed cells to NaCl	13±0.7*•	13±1.2	15±0.5*•	14.5±0.5*•
HP exposed cells to NaCl + HP	13±0.7*•	13.5±1.2	18±0.7*•	17.5±0.6*•
(B)				
Control	5.1±0.4	7.5±0.6	14±0.5	12±0.5
HP	5.1±0.4	4.5±0.6*	10±0.5*	9±0.9*
NaCl	7.5±0.6*	4±0.3*	7.5±0.6*	7±0.5*
HP exposed cells to NaCl	4.5±0.6*•	5.5±0.4•	9±0.5*•	8.5±0.4*•
HP exposed cells to NaCl + HP	4.5±0.6*•	6.5±0.6*•	10±0.5•	9.5±0.3*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 3. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on K^+ content in rice callus of BR and GR_{11} varieties.

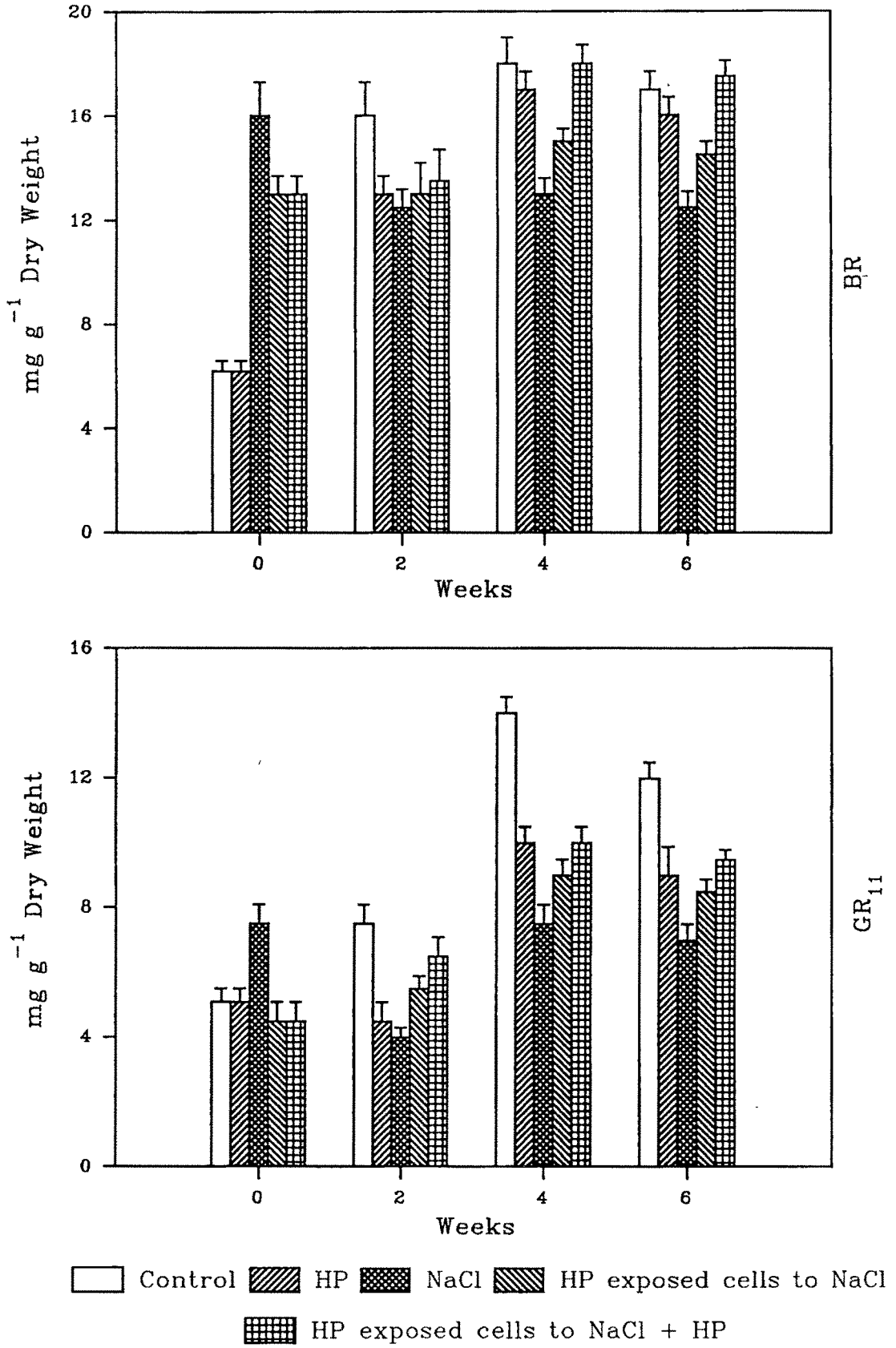


Table 6 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of Cl^- (mg g^{-1}dry wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@10.1±1.2	10.7±1.1	11.6±0.6	10.8±0.5
HP	10.1±1.2	9.2±1.8	10.9±1.2	9.8±0.5*
NaCl	10.7±1.1	45.6±3.5*	47.6±2.0*	47.1±1.5*
HP exposed cells to NaCl	9.2±1.8	40.2±2.1*	45.2±1.0*	44±1.1*•
HP exposed cells to NaCl + HP	9.2±1.8	39.9±1.3*•	40.2±3.5*•	38.8±1.6*•
(B)				
Control	11.1±1.3	11.5±1.4	12±1.4	11.8±0.5
HP	11.1±1.3	10.9±0.7	11.2±1.4	11.0±1.0
NaCl	11.5±1.4	55.3±2.7*	61.2±1.7*	59.9±1.7*
HP exposed cells to NaCl	10.9±0.7	51.8±1.2*	55.2±3.2*•	54.4±2.1*•
HP exposed cells to NaCl + HP	10.9±0.7	48.3±2.1*•	50.1±1.2*•	49.3±1.3*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 4. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on Cl^- content in rice callus of BR and GR_{11} varieties.

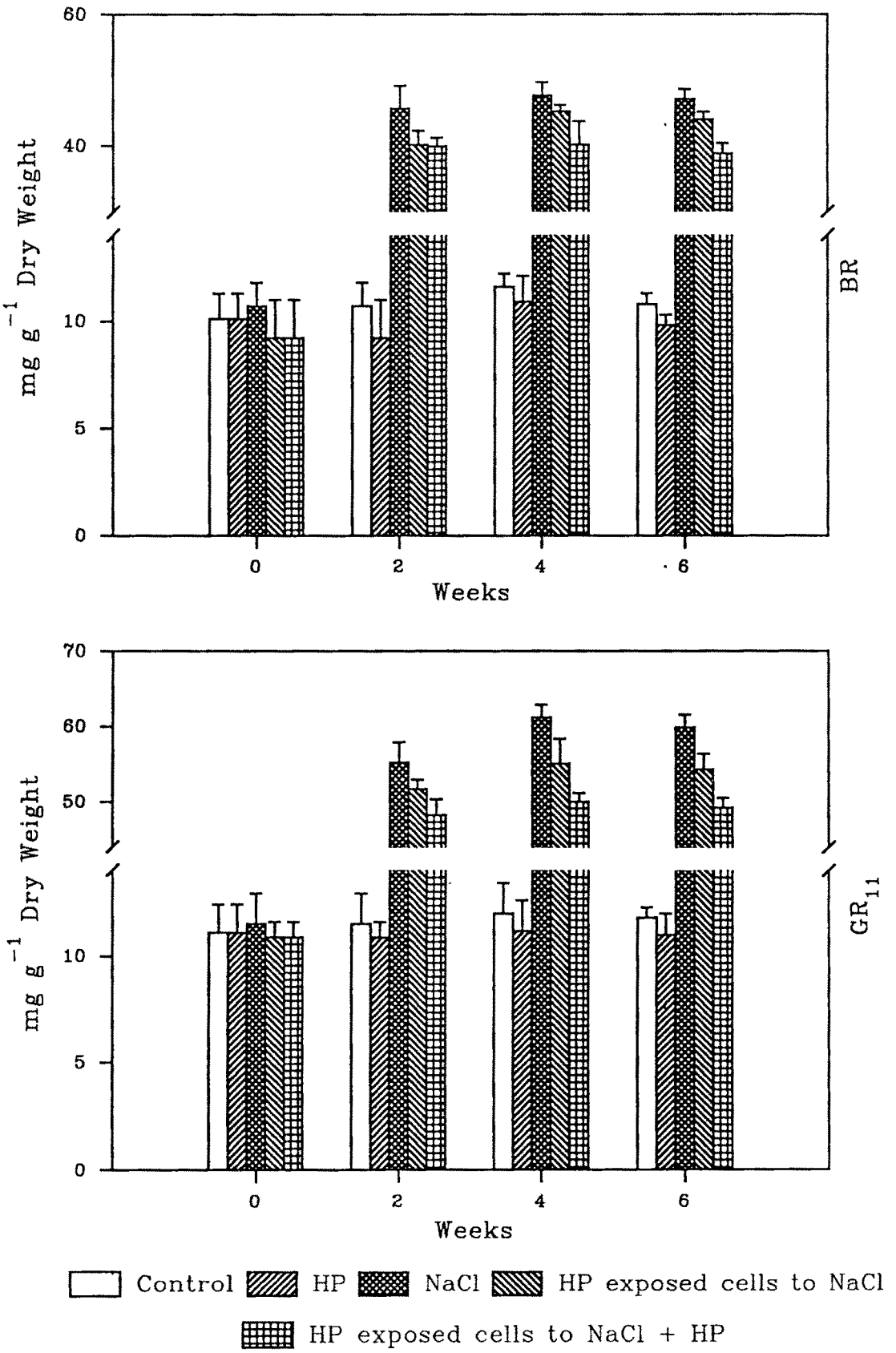


Table 7 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of Mg^{2+} (mg g^{-1}dry wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@14.4±0.3	10.4±0.4	20.4±0.4	17.5±0.4
HP	14.4±0.3	13.3±0.3*	24.5±0.4*	22.7±0.3*
NaCl	10.4±0.4*	30.6±0.4*	38.9±0.5*	34 0±0.5*
HP exposed cells to NaCl	13.3±0.3•	33.9±0.7*•	39.2±0.6*	38.0±13.5*
HP exposed cells to NaCl + HP	13.3±0.3•	40.9±0.7*•	43.3±0.5*•	41.6±0.6*•
(B)				
Control	11.4±0.3	10.2±0.3	16.4±0.4	16.0±0.4
HP	11.4±0.3	13.8±0.4*	20.5±0.5*	19.6±0.4*
NaCl	10.2±0.3*	20.6±0.4*	22.5±0.4*	21.5±0.5*
HP exposed cells to NaCl	13.8±0.4*•	25.6±0.4*•	31.0±0.6*•	30.2±0.7*•
HP exposed cells to NaCl + HP	13.8±0.4*•	30.0±0.3*•	37.2±0.7*•	36.0±0.7*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 5. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on Mg^{2+} content in rice callus of BR and GR_{11} varieties.

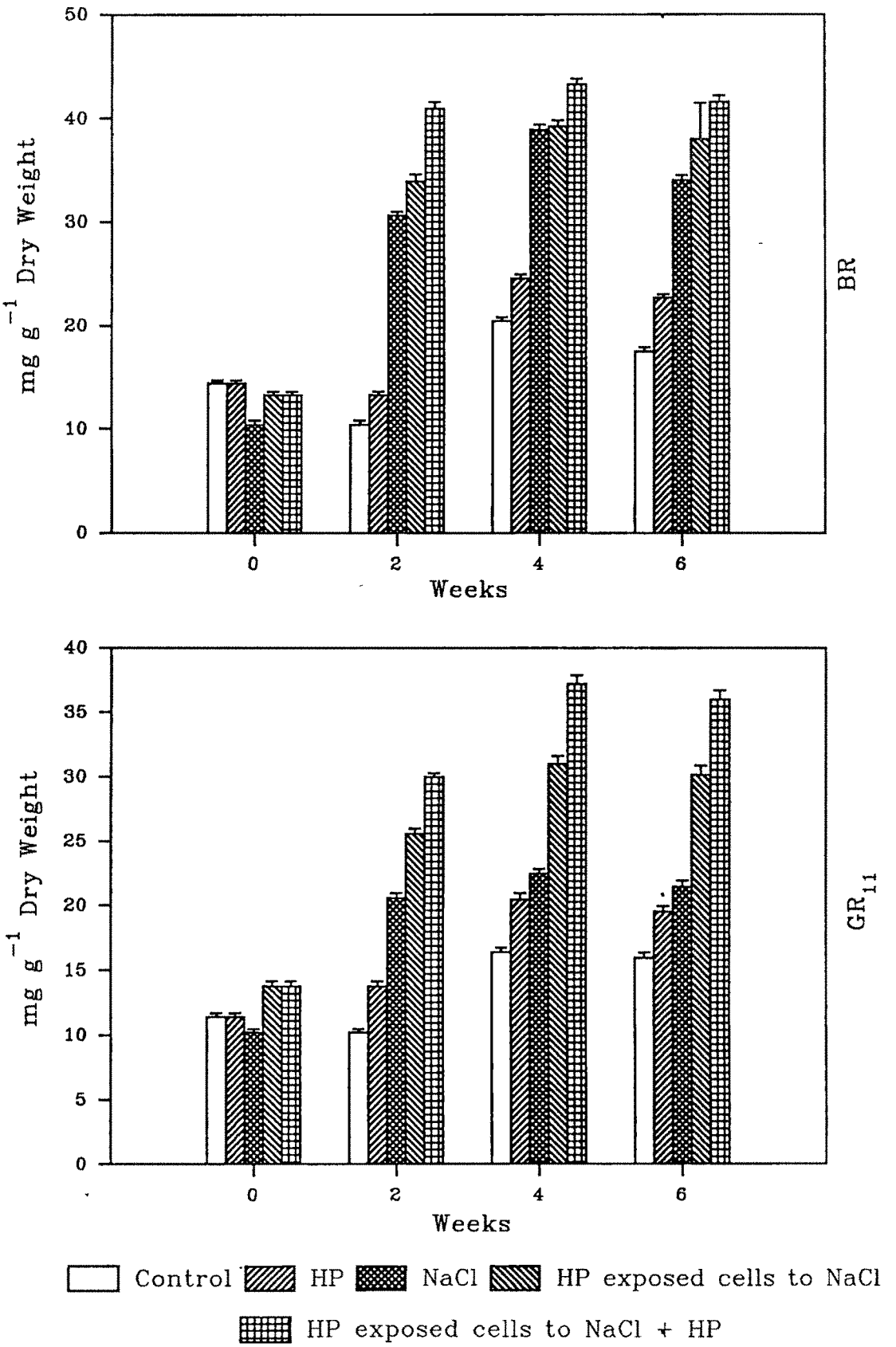


Table 8 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of Ca^{2+} (mg g^{-1}dry wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@8.57±1.3	20.0±1.0	25.96±1.6	19.31±0.9
HP	8.57±1.3	29.51±2.0*	31.0±1.0*	27±1.0*
NaCl	20.0±1.0*	23.93±1.4*	48.2±1.5*	44.0±0.9*
HP exposed cells to NaCl	29.51±2.0*•	30.98±4.9*•	57.9±1.3*•	52.2±0.8*•
HP exposed cells to NaCl + HP	29.51±2.0*•	40.2±2.1*•	74.2±1.5*•	65.3±1.1*•
(B)				
Control	5.28±0.7	6.90±0.4	11.6±0.9	10.0±0.8
HP	5.28±0.7	7.70±0.7	13.5±0.6*	11.12±0.5*
NaCl	6.9±0.4*	10.0±0.7*	20.98±1.1*	20.0±0.7*
HP exposed cells to NaCl	7.7±0.7*	14.5±1.1*•	26.0±0.7*•	24.0±0.3*•
HP exposed cells to NaCl + HP	7.7±0.7*	20.1±0.6*•	31.4±0.9*•	30.1±0.8*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 6. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on Ca^{2+} content in rice callus of BR and GR_{11} varieties.

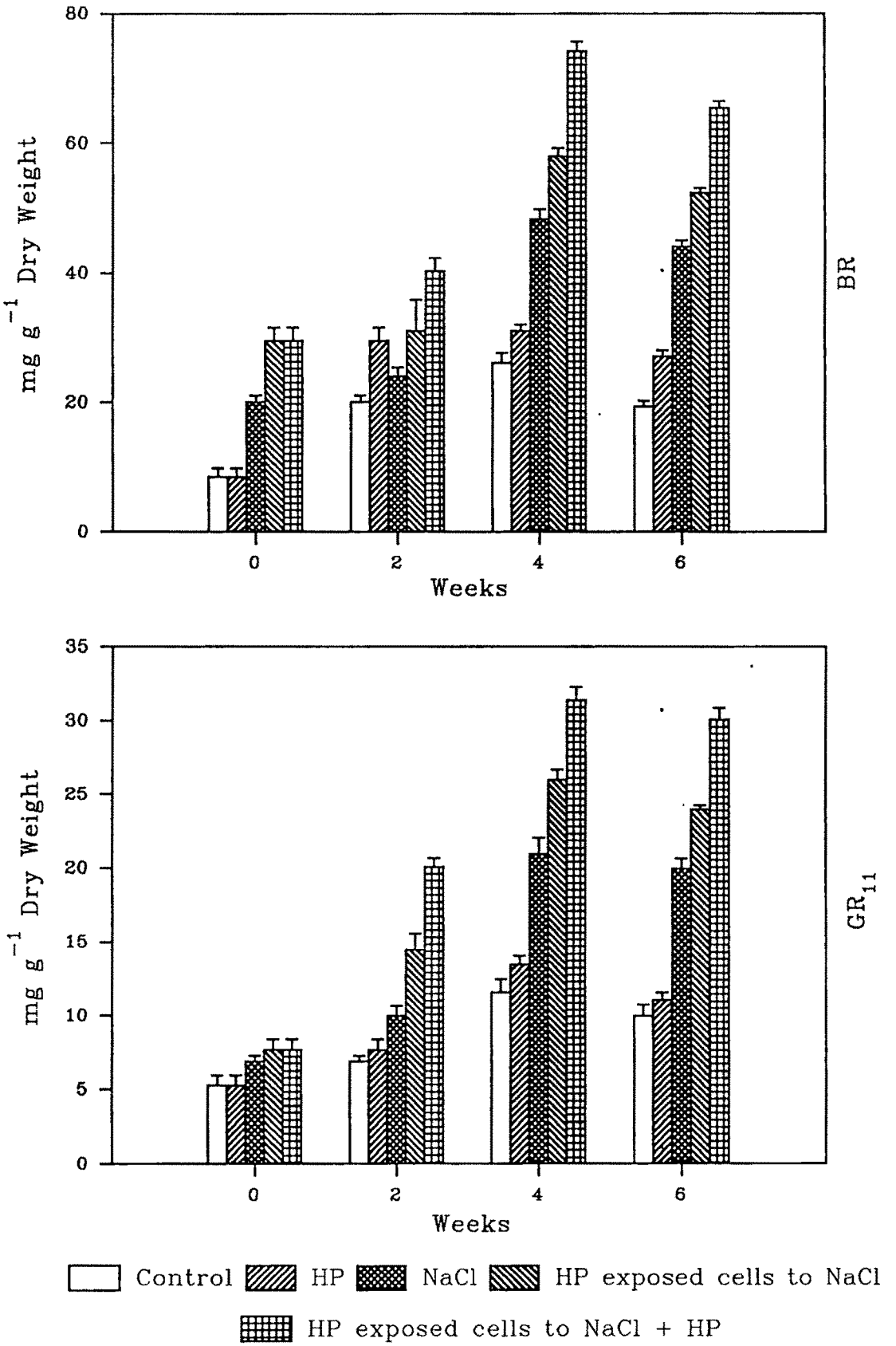


Table 9 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the free proline content (mg g^{-1} fr. wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@4.8±0.3	1.8↑±0.4	7.5±0.7	9.1±0.5
HP	4.8±0.3	5.9±0.7*	21.2±0.4*	27.5±0.6*
NaCl	1.8±0.4*	2.8±0.3*	15±1.1	18.3±1.0
HP exposed cells to NaCl	5.9±0.7*•	3.5±0.3•	35.24±0.9*•	38.1±0.8*•
HP exposed cells to NaCl + HP	5.9±0.7*•	4.3±0.5*•	39.66±1.2*•	43.4±1.4*•
(B)				
Control	5.6±0.2	2.9±0.6	3.9±0.5	4.4±0.6
HP	5.6±0.2	6±0.4*	6.6±0.3*	9±1.2*
NaCl	2.9±0.6*	2.9±0.3*	14.4±0.2*	14.8±1.0*
HP exposed cells to NaCl	6±0.4*•	3.5±0.7	18.5±0.8*•	20.3±0.6*•
HP exposed cells to NaCl + HP	6±0.4*•	4.1±0.3•	22.5±1.4*•	26.6±0.8*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 7. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on free proline content in rice callus of BR and GR_{11} varieties.

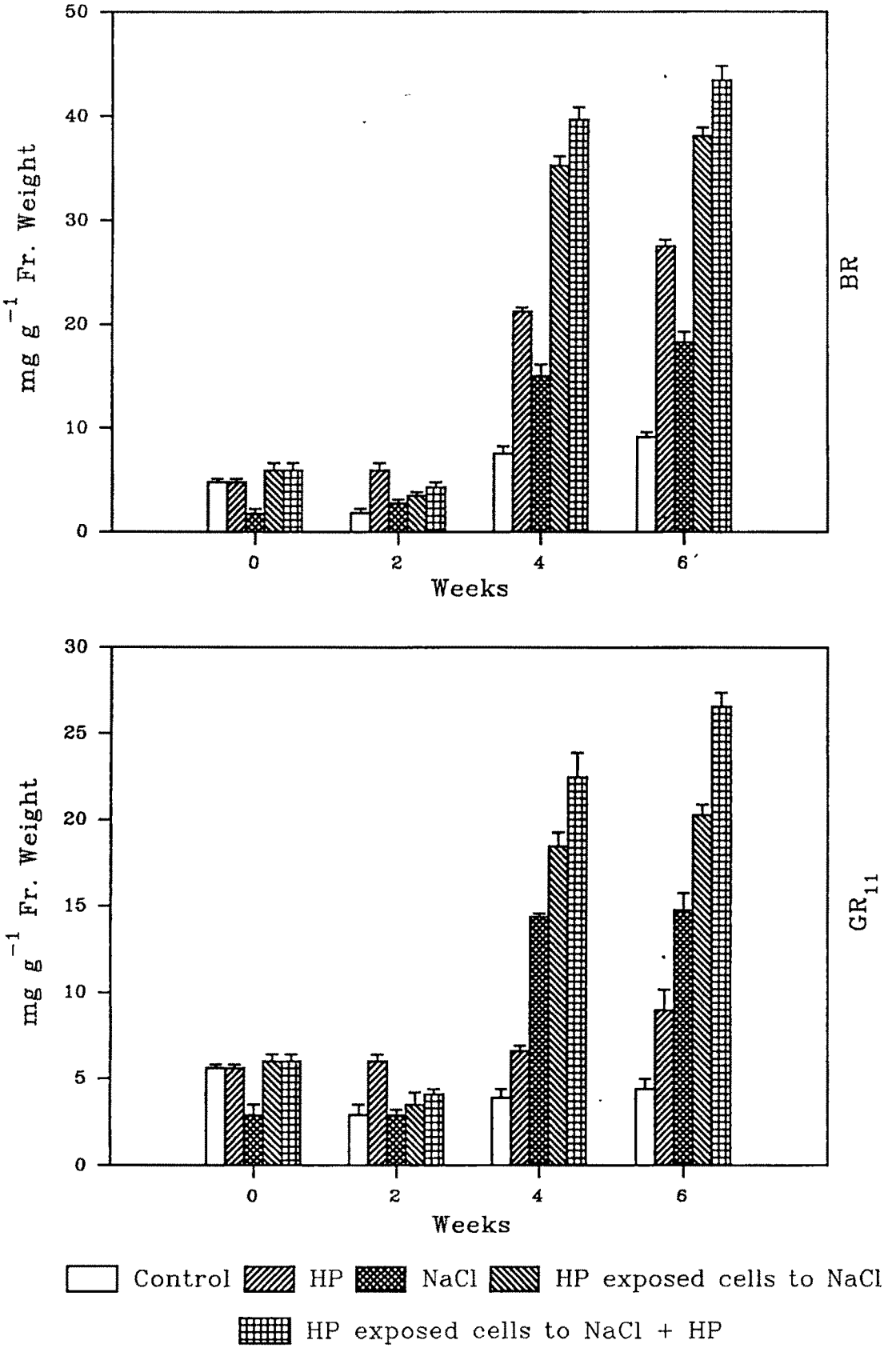


Table 10 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of proline oxidase (units mg^{-1} protein) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@5.0±1.0	14±1.3	11.1±0.8	9.1±0.9
HP	5.0±1.0	6.4±0.7*	6.0±0.9*	5.0±0.8*
NaCl	14.0±1.3*	8.9±1.1	8.0±0.9	5.4±0.6*
HP exposed cells to NaCl	6.4±0.7*•	7.6±0.9*	6.1±1.0*•	4.5±0.6*•
HP exposed cells to NaCl + HP	6.4±0.7*•	6.8±0.8*•	5.8±0.7*•	4.0±0.6*•
(B)				
Control	7.3±0.8	25.0±1.9	21.2±1.2	18±1.3
HP	7.3±0.8	19±1.1*	18.4±1.3*	16.9±1.7
NaCl	25±1.9*	22±1.5	17.5±0.9*	15.3±1.4*
HP exposed cells to NaCl	19±1.1*•	18.2±1.2*•	15.2±0.9*•	12.8±1.5*
HP exposed cells to NaCl + HP	19±1.1*•	15.0±1.1*•	12.5±1.2*•	11.8±1.6*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 8. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on activity of proline oxidase in rice callus of BR and GR_{11} varieties.

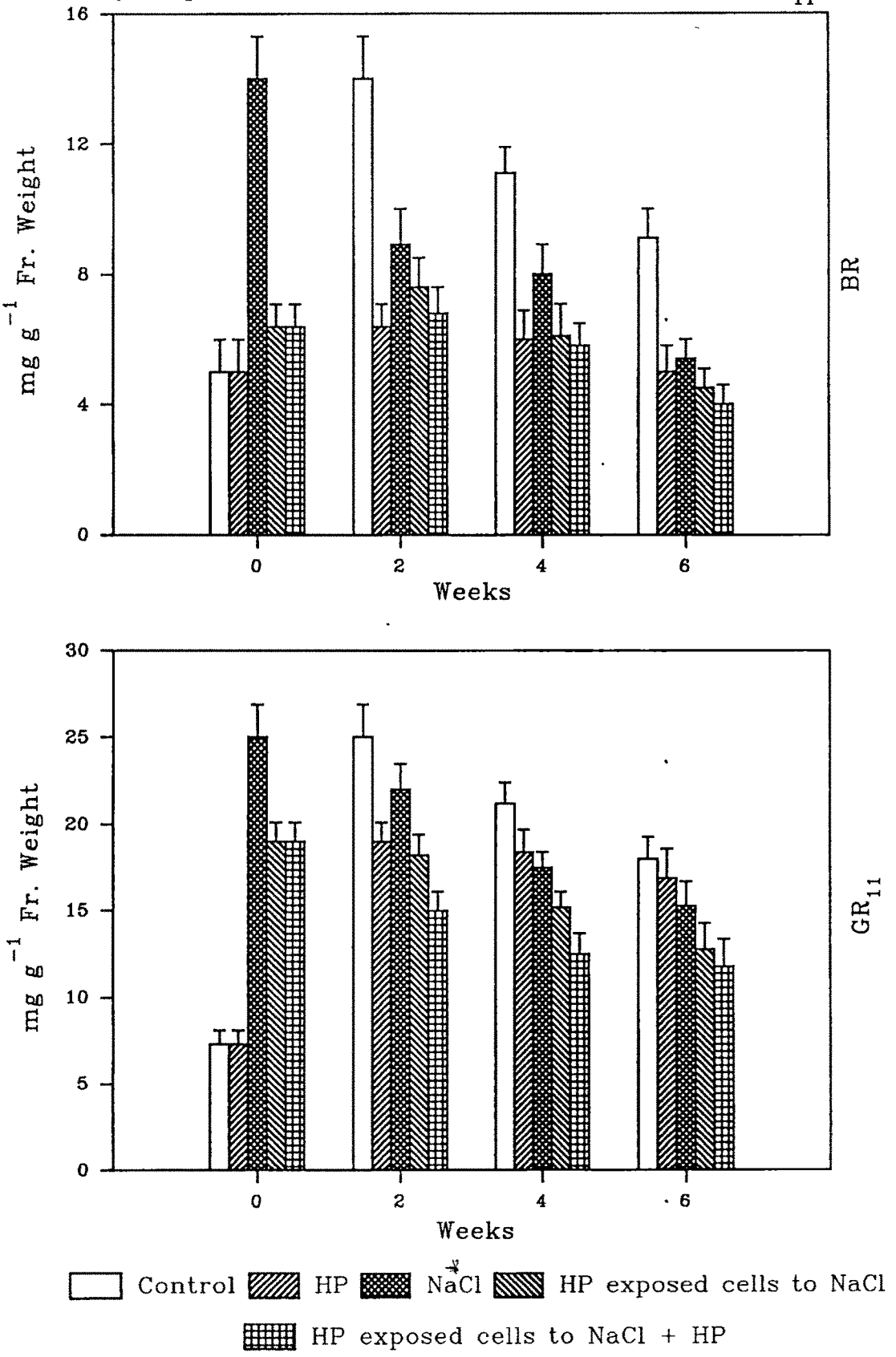


Table 11 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the total protein content (mg g^{-1} fr. wt.) of rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@4.78±0.6	1.60±0.3	5.30±0.5	8.2±0.9
HP	4.78±0.6	3.15±0.4*	7.2±0.4*	31.4±2.7*
NaCl	1.6±0.3*	1.81±0.2*	3.15±0.6*	5.3±0.2*
HP exposed cells to NaCl	3.15±0.4•	3.1±0.1*•	4.6±0.2•	6.86±0.5*•
HP exposed cells to NaCl + HP	3.15±0.4•	3.3±0.1*•	4.8±0.2•	7.7±0.4*•
(B)				
Control	4.5±0.2	2.31±0.4	5.26±1.0	16.58±0.9
HP	4.5±0.2	1.39±0.1*	9.43±0.6*	31.8±1.0*
NaCl	2.31±0.4*	2.10±0.3	2.95±0.3*	4.11±0.5*
HP exposed cells to NaCl	1.39±0.1*•	2.30±0.1*	3.3±0.4*	5.6±0.2*•
HP exposed cells to NaCl + HP	1.39±0.1*•	2.50±0.2*•	3.41±0.1*	6.5±0.2*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 9. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on total protein content in rice callus of BR and GR_{11} varieties.

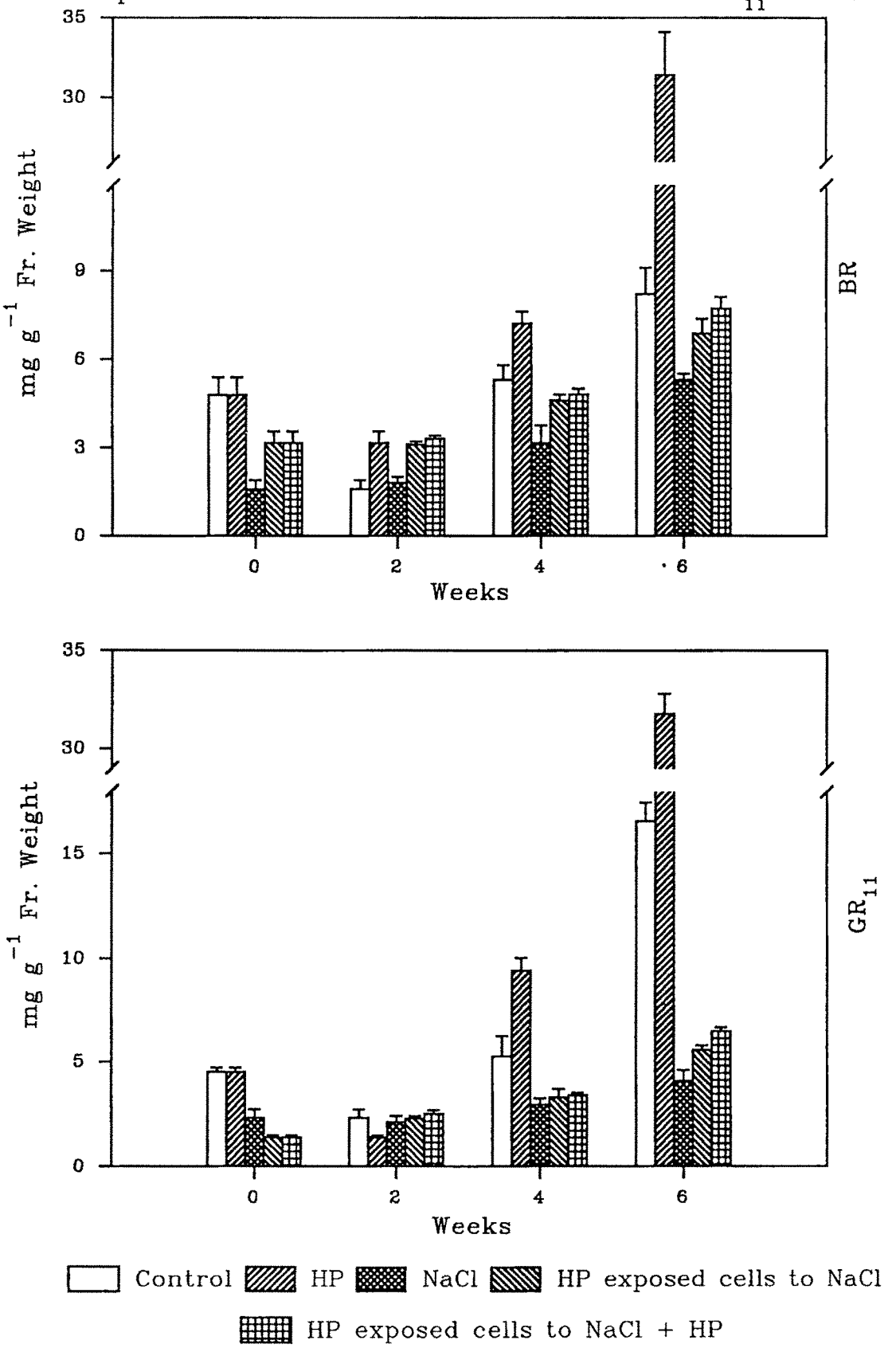


Table 12 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of putrescine (nmol g^{-1} fr.wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@162±6.1	140±9.3	153±10.6	150±14.8
HP	162±6.1	151±9.6*	155±11.3	152±9.3
NaCl	140±9.3*	153±5.4*	189±10.6*	185±13.5*
HP exposed cells to NaCl	151±9.6	136±7.3•	175±11.3	168±13.6•
HP exposed cells to NaCl + HP	151±9.6	128±8.1*•	168±11.5	165±9.8
(B)				
Control	195±9.6	185±6.0	253±15.8	249±10.6
HP	195±9.6	200±6.5*	255±9.4	253±10.4
NaCl	185±6.0	250±12.2*	330±17*	326±8.5*
HP exposed cells to NaCl	200±6.5•	248±9.1*	300±18.4•	295±11.6*•
HP exposed cells to NaCl + HP	200±6.5•	241±10.3*	270±15.5*•	263±10.6*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 10. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on putrescine content in rice callus of BR and GR_{11} varieties.

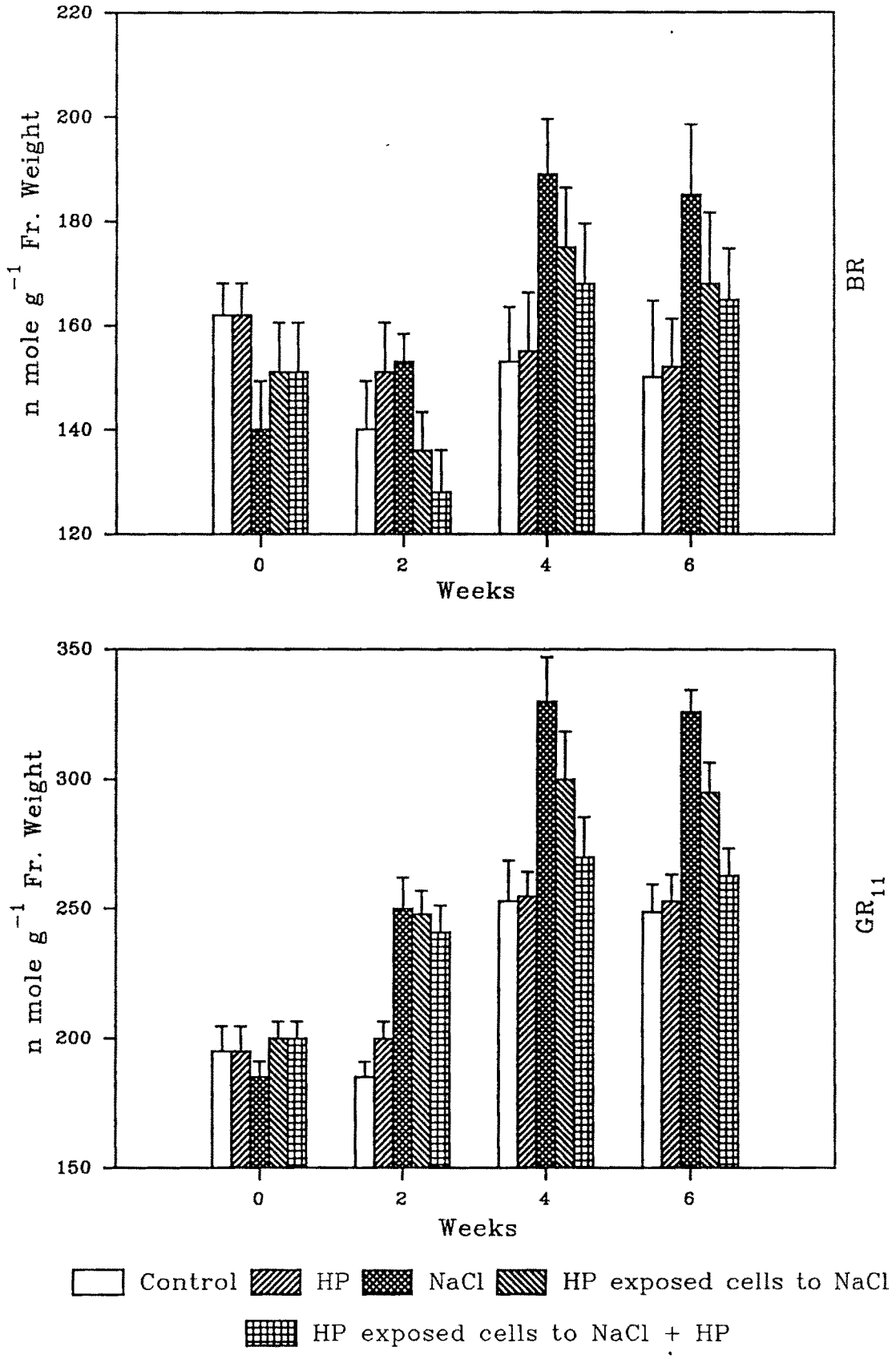


Table 13 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of spermine ($nmolg^{-1}$ fr. wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@120±11.9	100±8.4	168±16.1	160±15.4
HP	120±11.9	195±7.6*	310±14.9*	285±15.4*
NaCl	100±8.4*	89±15.7*	152±12.4*	146±12.6*
HP exposed cells to NaCl	195±7.6*•	131±9.5•	240±15.2*•	228±13.8*•
HP exposed cells to NaCl + HP	195±7.6*•	149±13.0*•	301±13.0*•	289±17.0*•
(B)				
Control	90±9.1	75±7.2	142±15.6	135±10.8
HP	90±9.1	110±11.9*	200±12.2*	193±19.1*
NaCl	75±7.9*	55±9.4*	82±8.1*	80±9.3*
HP exposed cells to NaCl	110±11.2*•	85±11.0•	120±15.0*•	110±12.2*•
HP exposed cells to NaCl + HP	110±11.2*•	100±8.6*•	128±9.7*•	118±13.7*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 11. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on spermine content in rice callus of BR and GR_{11} varieties.

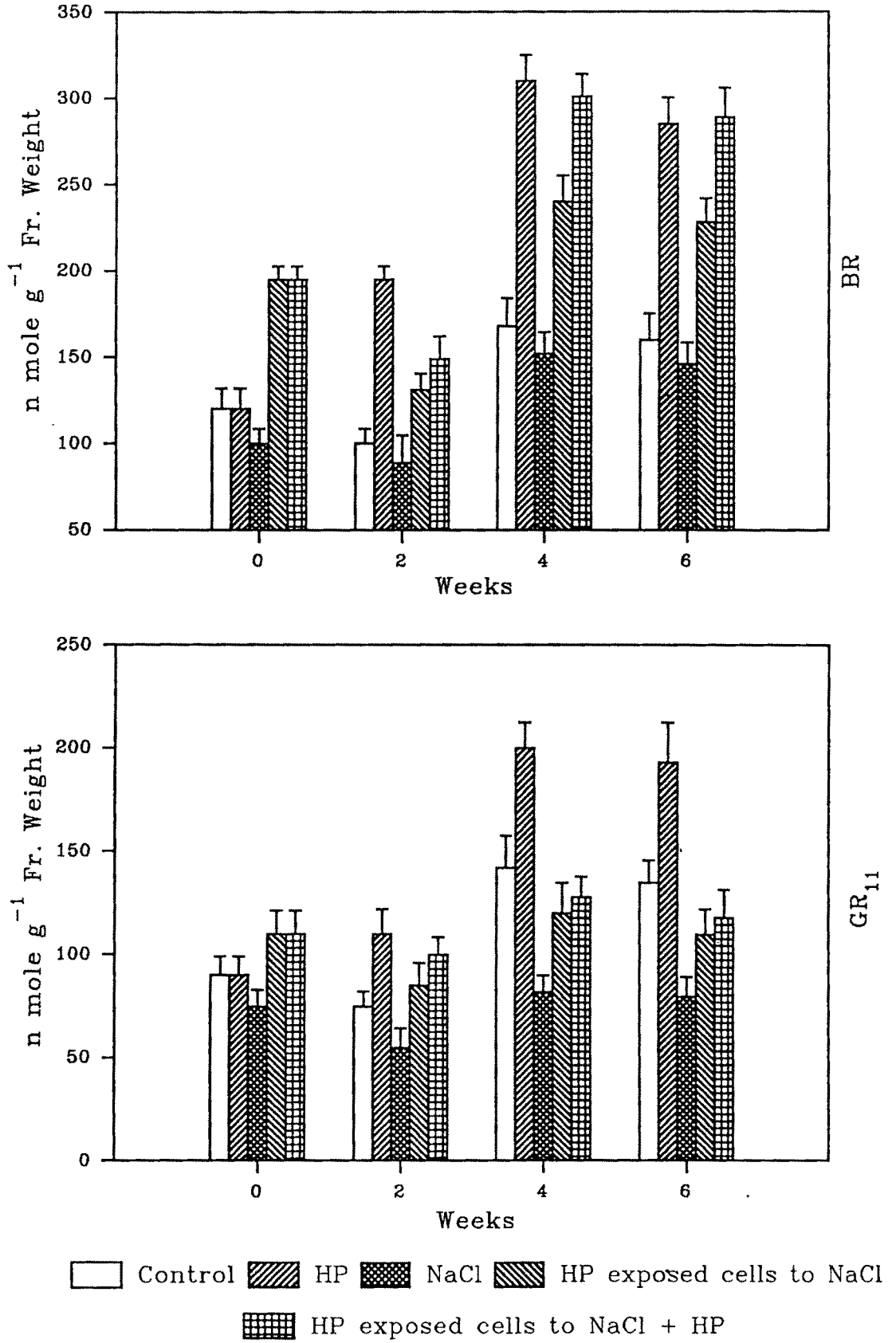


Table 14 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the content of spermidine (nmolg^{-1} fr. wt.) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@201 ± 14	174 ± 13.1	231 ± 13.3	223 ± 8.2
HP	201 ± 14	185 ± 12.6*	290 ± 14.2*	280 ± 9.8*
NaCl	174 ± 13.1*	125 ± 16.0*	189 ± 14.2*	185 ± 6.5*
HP exposed cells to NaCl	185 ± 12.6	180 ± 14.2*•	260 ± 13.3*•	252 ± 6.8*•
HP exposed cells to NaCl + HP	185 ± 12.6	208 ± 12.0*•	298 ± 12.6*•	290 ± 7.6*•
(B)				
Control	120 ± 11.8	105 ± 11.3	165 ± 12.1	152 ± 7.1
HP	120 ± 11.8	129 ± 13.5*	181 ± 8.8*	170 ± 7.5*
NaCl	105 ± 11.3*	67 ± 9.1*	91 ± 7.5*	85 ± 6.7*
HP exposed cells to NaCl	129 ± 13.5•	75 ± 9.2*	115 ± 8.8*•	109 ± 6.3*•
HP exposed cells to NaCl + HP	129 ± 13.5•	89 ± 7.0•	135 ± 11.6•	130 ± 9.1•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 12. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on spermidine content in rice callus of BR and GR_{11} varieties.

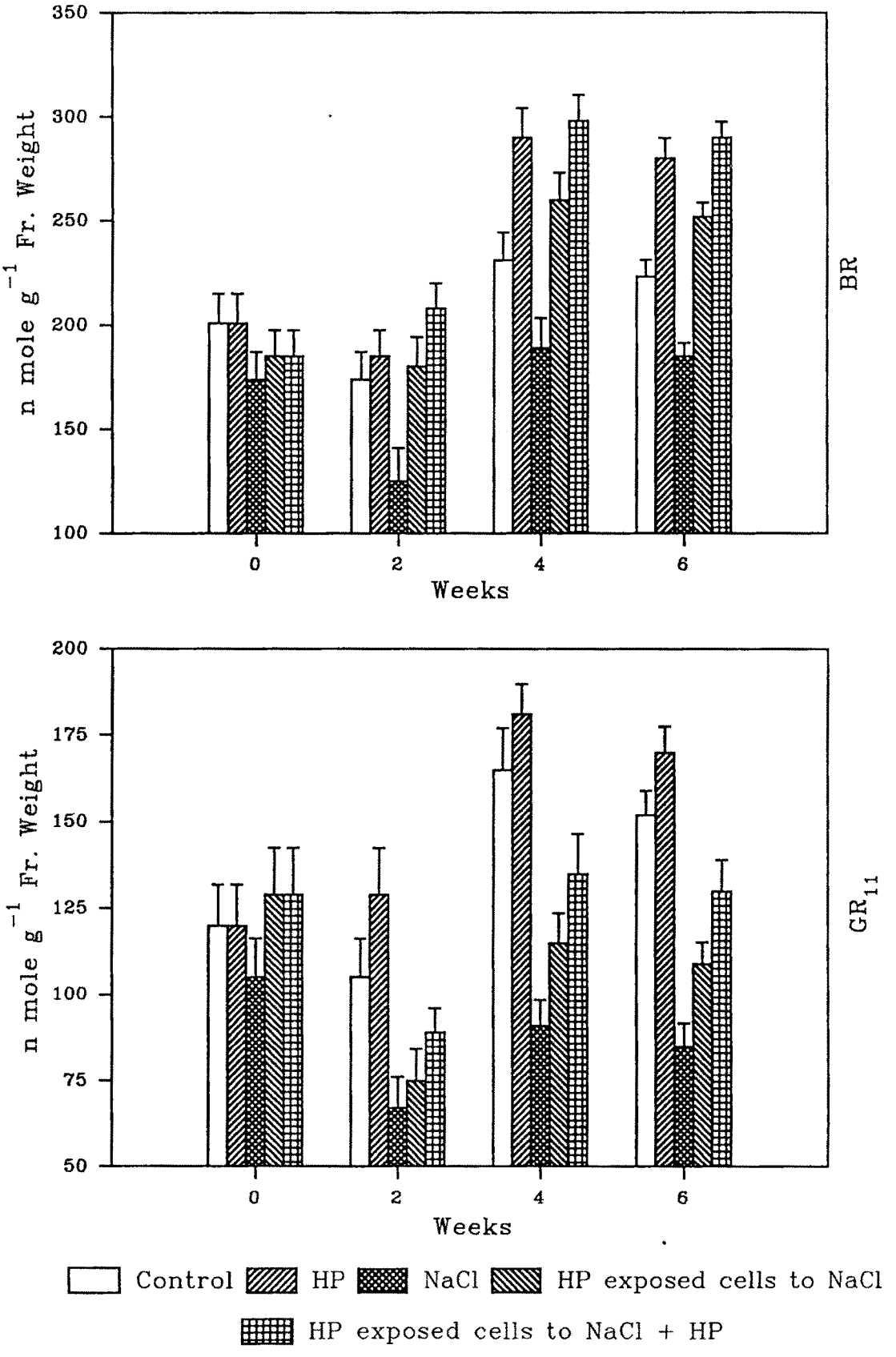


Table 15 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of IAA oxidase (units mg^{-1} protein) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@8.6±0.6	9.64±0.8	7.18±0.8	8.1±0.2
HP	8.6±0.6	8.9±1.0	5.67±0.8	8.75±0.9
NaCl	9.64±0.8	10.21±0.8*	8.9±0.9*	9.6±0.4*
HP exposed cells to NaCl	8.9±1.0	9.5±0.5	7.9±0.5*•	8.89±0.3•
HP exposed cells to NaCl + HP	8.9±1.0	8.9±0.8	5.86±0.6*•	7.2±0.5*•
(B)				
Control	3.5±0.4	5.85±0.1	3.38±0.1	6.26±0.3
HP	3.5±0.4	6.5±1.3	4.8±0.7*	6.3±0.4
NaCl	5.85±0.1*	7.9±0.8*	6.5±0.4*	8.9±0.6*
HP exposed cells to NaCl	6.5±1.3*	7.1±0.4*	6.3±1.3*	8.1±0.3*•
HP exposed cells to NaCl + HP	6.5±1.3*	6.3±1.3	5.9±0.5*	7.8±0.8

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 13. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on IAA oxidase activity in rice callus of BR and GR_{11} varieties.

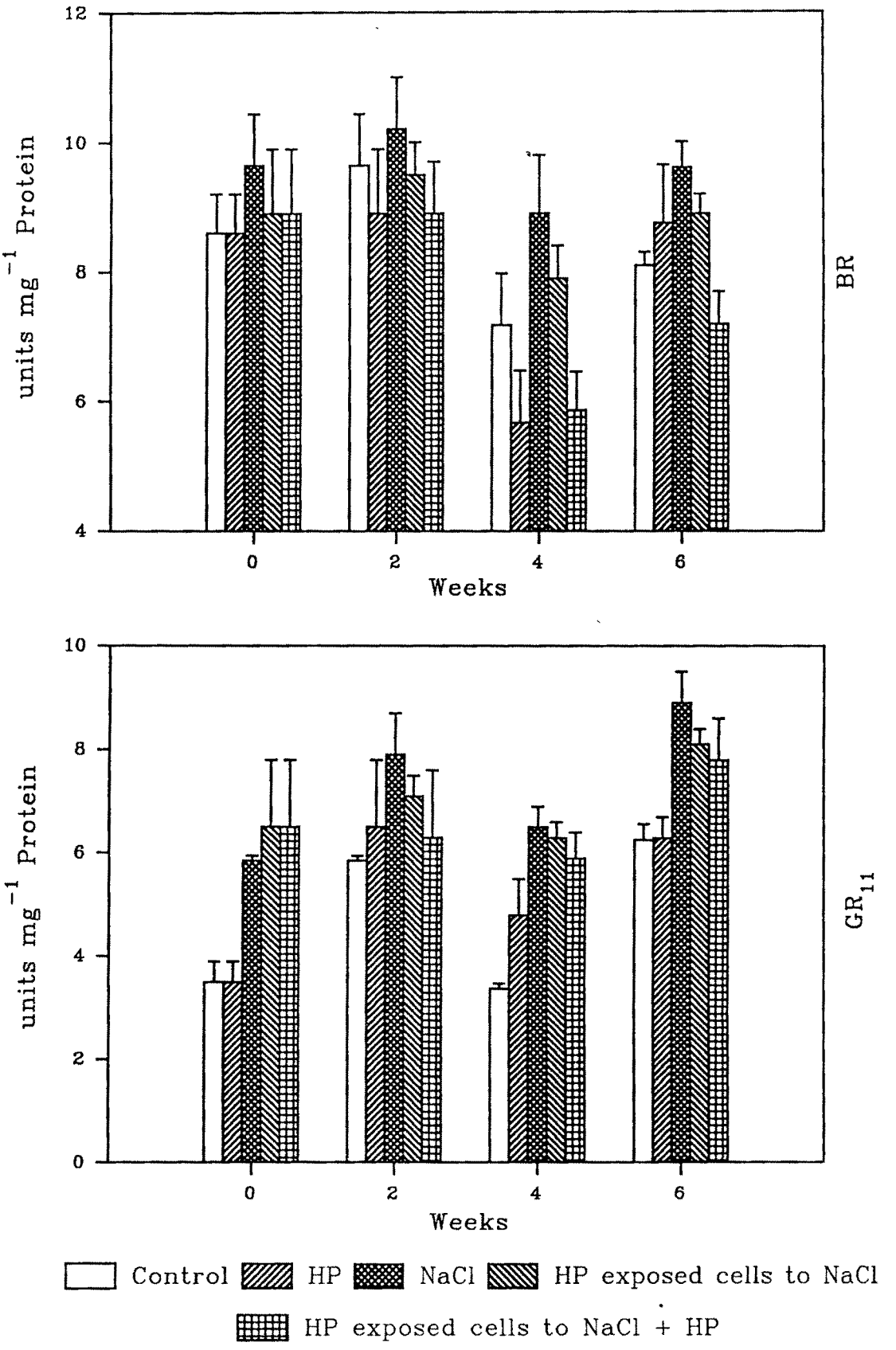


Table 16 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of amylase (units mg^{-1} protein) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@12.22±1.6	38.29±2.8	62.98±1.1	27.96±0.7
HP	12.22±1.6	61.58±1.8*	71.21±0.9*	28.52±0.9
NaCl	38.29±2.8*	9.83±1.4*	19.36±0.7*	14.36±0.7*
HP exposed cells to NaCl	61.58±1.8*•	12.5±1.0*•	22.63±1.4*•	17.69±0.5*•
HP exposed cells to NaCl + HP	61.58±1.8*•	27.6±0.9*•	30.18±0.9*•	24.33±1.0*•
(B)				
Control	11.84±0.6	18.95±1.4	61.69±1.2	16.1±0.4
HP	11.84±0.6	72.95±1.3*	85.28±1.0*	19.33±1.3*
NaCl	18.95±1.4*	7.5±0.9*	10.2±0.8*	7.09±0.1*
HP exposed cells to NaCl	72.95±1.3*•	8.26±0.7*	13.81±0.8*•	8.62±0.3*•
HP exposed cells to NaCl + HP	72.95±1.3*•	9.45±0.6*•	16.24±0.9*•	9.95±0.9*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 14. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM on amylase activity in rice callus of BR and GR_{11} varieties.

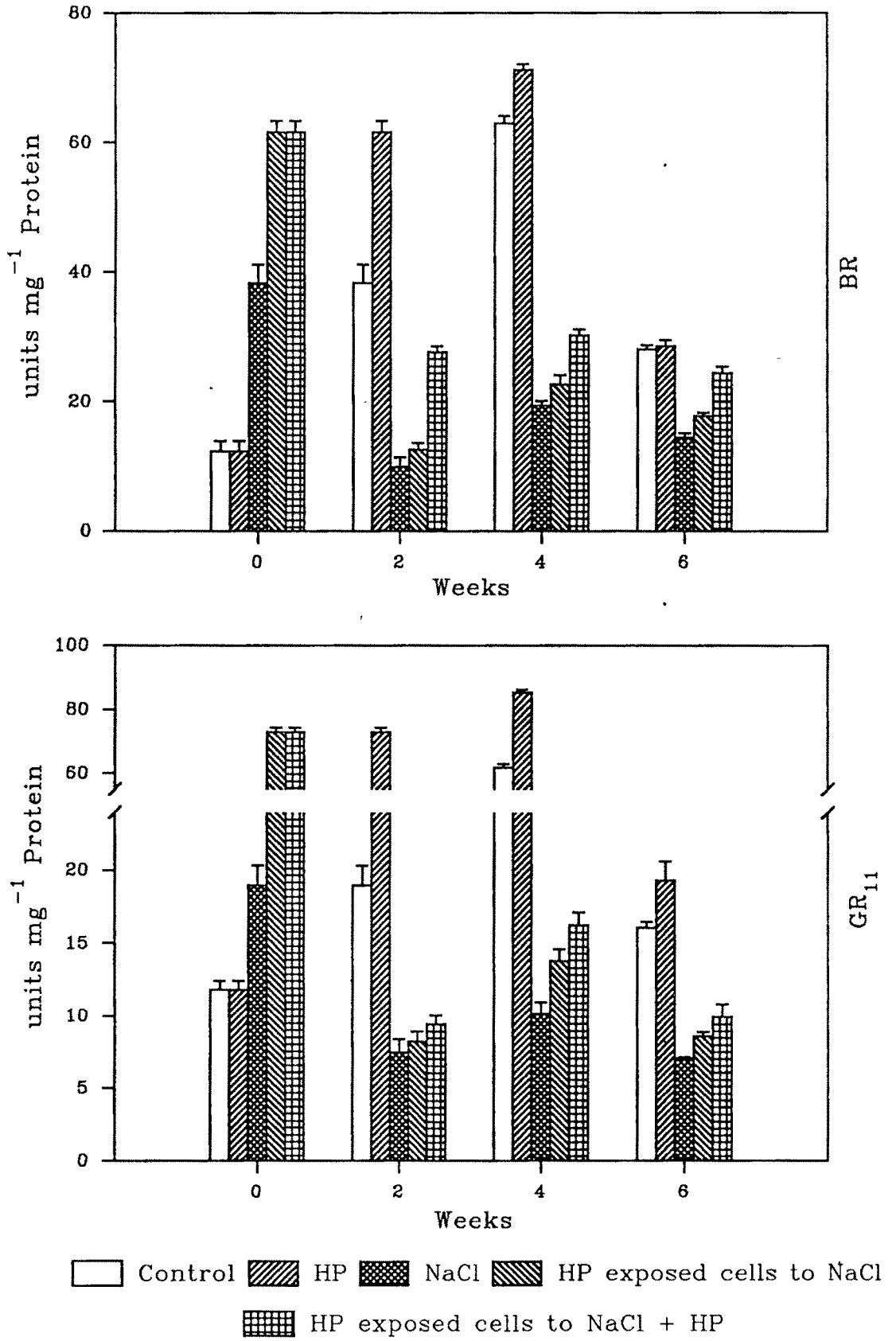


Table 17 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of invertase (units mg^{-1} protein) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@56.6±0.6	40.2±0.7	72.4±0.7	59±1.1
HP	56.6±0.6	52.6±0.9*	117.9±1.4*	73.6±1.0*
NaCl	40.2±0.7*	28.3±0.4*	41.5±0.7*	28±0.3*
HP exposed cells to NaCl	52.6±0.9*•	33.1±0.4*•	47.4±0.6*•	41.9±0.6*•
HP exposed cells to NaCl + HP	52.6±0.9*•	36.8±0.3*•	53.4±0.9*•	43.4±0.5*•
(B)				
Control	43.8±0.7	30.9±0.4	70.8±1.2	34±0.6
HP	43.8±0.7	39.6±0.5*	110.1±1.6*	51±0.7*
NaCl	30.9±0.4	23.5±0.5*	25.1±0.4*	15.6±0.4*
HP exposed cells to NaCl	39.6±0.5•	28.6±0.3*•	31.0±0.3*•	25.5±0.3*•
HP exposed cells to NaCl + HP	39.6±0.5•	29.9±0.3*•	39.2±0.5*•	32.8±1.1*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 15. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on invertase activity in rice callus of BR and GR_{11} varieties.

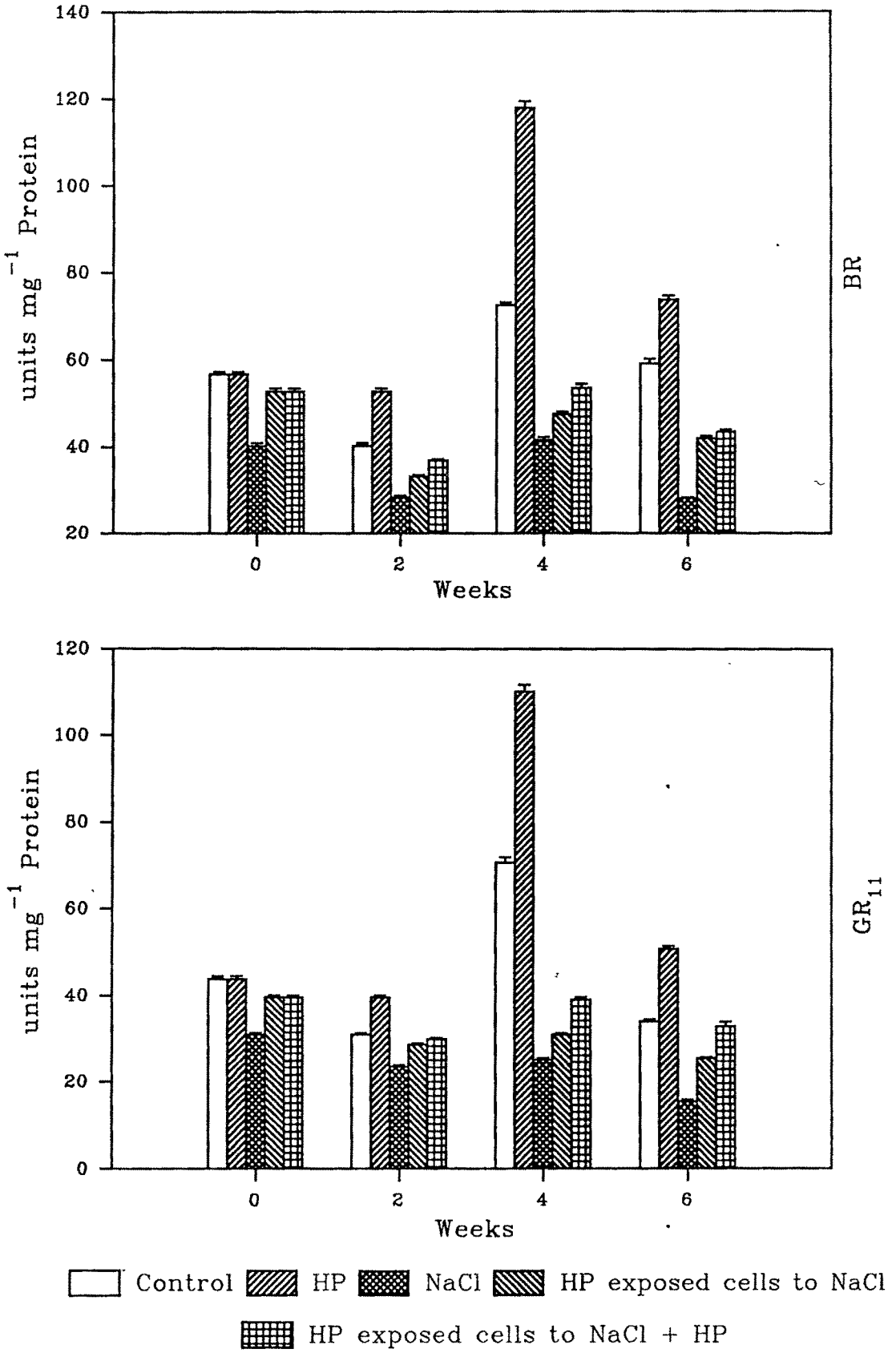


Table 18 : Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of cellulase (units mg^{-1} protein) in rice callus of BR (A) and GR_{11} (B) varieties.

Treatments	Time Interval (weeks)			
	0	2	4	6
(A)				
Control	@33.1±0.6	46.9±1.2	51.6±1.7	49±1.5
HP	33.1±0.6	52.8±0.9*	98.4±1.4*	85.6±1.1*
NaCl	46.9±1.2*	12.1±0.4*	21.3±0.6*	16.4±0.7*
HP exposed cells to NaCl	52.8±0.9*•	24.0±0.7*•	30.8±1.0*•	24.3±0.8*•
HP exposed cells to NaCl + HP	52.8±0.9*•	32.0±0.6*•	34.7±0.8*•	30.3±0.9*•
(B)				
Control	35.8±0.5	40.4±1.2	48.8±1.0	38.1±1.0
HP	35.8±0.5	45.3±0.8*	53.1±0.7*	46.2±1.1*
NaCl	40.4±1.2*	14.4±0.8*	15.8±0.5*	9.8±0.6*
HP exposed cells to NaCl	45.3±0.8*•	18.2±0.5*•	20.5±0.5*•	12.1±0.7*•
HP exposed cells to NaCl + HP	45.3±0.8*•	21.4±0.6*•	26.8±0.9*•	24.7±0.6*•

* Values differ significantly ($P < 0.05$) compared to control

• Values differ significantly ($P < 0.05$) compared to NaCl

@ Values indicates ± Standard Deviation of three separate measurements

Fig. 16. Effect of hydroxyproline (HP) [10mM] and NaCl [200mM] on cellulase activity in rice callus of BR and GR_{11} varieties.