



## VARIATIONS IN ABSCISIC ACID, INDOLE-3-ACETIC ACID, GIBBERELIC ACID AND ZEATIN CONCENTRATIONS IN TWO BEAN SPECIES SUBJECTED TO SALT STRESS

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Common bean (*Phaseolus vulgaris* L.) is sensitive to drought and salinity, while an ancestral legume, tepary bean (*P. acutifolius* A. Gray) is cultivated successfully where high temperature and drought are common (Lazcano-Ferrat and Lovatt, 1999). Hence, *P. acutifolius* is a potential source of stress tolerant traits for *P. vulgaris* through interspecific hybrids. This study comparatively evaluated the effects of salt stress on leaf relative water content (RWC), soluble protein, the phytohormones indole acetic acid (IAA), gibberellic acid (GA<sub>3</sub>), zeatin, and abscisic acid (ABA) levels in *P. vulgaris* and *P. acutifolius*. With the exception of ABA, stress-induced changes in hormonal levels putatively related to stress tolerance have not been investigated previously in either species. Treatment with 50 mM, 100 mM and 150 mM NaCl reduced relative water and protein content in *P. vulgaris*, but did not affect relative water content and increased protein content in *P. acutifolius*. Varietal differences between *P. vulgaris* and *P. acutifolius* were also observed in hormonal content during the stress period. ABA levels in salt-treated plants of *P. vulgaris* increased but did not change in *P. acutifolius* versus the controls. Both IAA and gibberellin levels increased in leaves of *P. acutifolius* but decreased in *P. vulgaris* under salt stress. In salt-treated plants, zeatin concentrations in leaves of *P. vulgaris* decreased within 24–72 h after treatment. However, *P. acutifolius* responded to all tested NaCl levels by increasing zeatin concentrations after short-term NaCl treatment.

**Key words:** *Phaseolus*, relative water content, abscisic acid, indole acetic acid, gibberellin, zeatin, NaCl-stress, protein.

### INTRODUCTION

Soil salinity is a major abiotic stress in plant agriculture worldwide. This has led to research into the physiological and biochemical mechanisms underpinning salt tolerance, with the aim of improving crop plants. Plants have a centralized system of response, enabling them to respond to any adverse conditions regardless of the nature of the stress. The responses of plants to stress include growth inhibition, decreased nutrient uptake and lower photosynthetic rate. Plants have also been observed to respond to environmental extremes by changing the hormonal balance (Nilsen and Orcutt, 1996).

Whether water status, hormonal regulation or photosynthesis exerts the dominant control over growth of plants in dry or saline soils is an issue debated for the last two decades and not yet resolved. However, over a time scale of days there is much evidence to suggest that hormonal signals rather than water relations control growth in saline soils (Munns, 2002). Our understanding of hormone physiology under stress conditions is limited by the scant amount of information available.

In a number of plant species, marked and often rapid changes in hormonal levels are commonly observed in response to stress. For many years, abscisic acid (ABA) and to a lesser extent ethylene

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have captured the attention of most hormone physiologists interested in plant responses to abiotic stresses. It is now well known from research that ABA mediates many physiological responses to environmental stress. For example, abscisic acid content increased under the influence of dehydration (Hubick et al., 1986), salinity (Roeb et al., 1982; Sibole et al., 1998) and low and high temperature (Talanova and Titov, 1994), supporting the suggestion that this hormone actively participates in adaptive responses of the plants to different stress factors.

However, despite their importance, auxin, gibberellin and cytokinin physiology under stress conditions is poorly understood. Darbyshire (1971) found an increase in IAA oxidase activity in vitro with decreasing water potential, and correlated it with an observed decrease in endogenous auxin. The free or diffusible auxin content of etiolated *Zea mays* coleoptiles decreased under salinity stress (Naqvi et al., 1986). In contrast, Veselov et al., (2002) reported an increase in IAA content in the shoot of wheat seedlings under osmotic shock created by PEG (polyethylene glycol) treatment. Similarly, we observed enhanced levels of IAA in the leaves of salt-treated plants of wild tomato *Lycopersicon pennellii* (Yurekli et al., 2001). Very little is known about gibberellin levels, particularly under stress conditions. In sorghum, Amzallag et al. (1992) reported that gibberellic acid concentrations decreased with decreasing concentrations of salts in the growth medium. Aharoni and Richmond (1978) found that drought stress caused a decline in the level of gibberellin in tissue of lettuce leaves. Indirect evidence from studies on gladiolus also shows a reduction in gibberellin concentrations under water stress (Rao and Ram, 1986). However, Hubick et al. (1986) showed that neither total free gibberellin levels nor their distribution showed a change in drought-stressed, aeroponically grown sunflower plants. In the leaves of the salt-tolerant tomato species *L. pennellii* we observed an increase in gibberellin content under short-term salt stress (Yurekli et al., 2001).

Despite their role in plant response to stress, information about how endogenous cytokinins are affected is limited and contradictory. Cytokinin levels tend to decrease under adverse environmental conditions. Previously it was generally thought that a reduction of cytokinins from the root alters gene expression in the shoot and thereby elicits appropriate responses to ameliorate the deleterious effects of stress. However, recent studies have indicated that

transcription of stress-inducible genes can also be caused by cytokinin application, indicating possible roles for cytokinins in affecting the overall hormonal balance following the imposition of environmental stress, and thereby playing a role in systemic responses to stress (Hare et al., 1997). Related to this, particular attention has been devoted to the interactions of cytokinins with ABA, ethylene, salicylic acid and jasmonates. On the other hand, there has been little research to date on the effects of salt stress on IAA, gibberellin and cytokinin levels in different genotypes showing different sensitivity to the stress.

Beans, which are good source of proteins, vitamins and minerals, are basic foodstuffs particularly in tropical and subtropical areas. However, their production and availability are not enough to fill the requirements of human consumption because of the increase in the area of arid zones, and adverse climatic and agronomic conditions such as drought and salinity (Camacho-Barron and Gonzales De-Mejia, 1998). Stansell and Smittle (1980) reported that salt stress during any stage of growth seriously hampers the productivity of beans. As is the case for many other crops, however, differences in drought and salt tolerance have also been reported among the diverse species of the genus *Phaseolus* (Lazcano-Ferrat and Lovatt, 1999). The common bean (*P. vulgaris*) is sensitive to drought and salinity. Hence its glyco-phytic character limits its growth in saline environments and creates the need for selection of more tolerant bean varieties that can be recommended for cultivation under adverse environmental conditions.

On the other hand, an ancestral legume, *P. acutifolius*, which has been consumed by natives for thousand of years, is cultivated successfully where high temperatures and drought are common (Frederici et al., 1990). *P. acutifolius* is a potential source of stress-tolerant traits for *P. vulgaris* obtainable through interspecific hybrids (Haghigi and Ascher, 1988). Understanding the physiological and biochemical mechanisms conferring drought and salinity tolerance to this species is very important for the development of selection and breeding strategies. In previous studies, some attempts have been made to evaluate physiological and biochemical changes, including ion exchange and the role of solutes such as proline in osmotic adjustment to high temperature, drought and salinity in *P. acutifolius* and *P. vulgaris* (Sibole et al., 1998; Camacho-Barron and Gonzales De-Mejia, 1998). To the best of our knowledge there has been no study of the involvement of endogenous hormones such as ABA, IAA,

gibberellins and cytokinins in different plant tolerance strategies developed against drought and salinity. The difference in drought and salt tolerance between these bean species cannot be related only to the effect of ions or nutritional disturbances, but may be related to some other factors that reduce the net assimilation rate of *P. vulgaris* during the early stages of growth. Since the osmotic rather than the ionic effect of salt occurs within days, a possible factor regulating the growth of salt-stressed plants is abscisic acid. It was previously reported that bean species show differential drought stress tolerance at their various growth stages.

In the present study we investigate the effect of different periods of salt stress at different stages of plant growth on leaf relative water content, soluble protein, abscisic acid, indole-3-acetic acid, gibberellin and zeatin levels in two bean species, to provide information on the physiological basis of the differences in the sensitivity of the cultivars to salt stress.

## MATERIALS AND METHODS

*Phaseolus vulgaris* L. and *Phaseolus acutifolius* A. Gray were used in this study. *P. vulgaris* and *P. acutifolius* seeds were obtained from Professor J.G. Waines (University of California, Riverside CA, U.S.A.). Seeds were germinated on filter paper in Petri dishes and incubated in Hoagland medium (Hoagland and Arnon, 1938). The Petri dishes were kept at 25°C for 3 days in the dark. Seedlings were transferred to plastic pots containing perlite, and then grown in a plant growth chamber at 24 ± 2°C with a 15 h photoperiod (220 W m<sup>-2</sup>; 90% cool white fluorescent and 10% incandescent bulbs) under 65% relative humidity for 40 days. The plants were grouped in three pots of 18 plants for each experimental treatment group and approximately 2 g leaves of bean seedlings were harvested and used for protein, RWC and hormone analysis. The plants were then exposed to salinity stress at three different NaCl concentrations (50 mM, 100 mM and 150 mM) for 24, 48 and 72 h. Relative water content (RWC) was measured according to Perl-Treves and Galun (1991). Protein concentration was determined according to Bradford (1976) method.

Extraction and purification of ABA, IAA, GA<sub>3</sub> and zeatin were according to Yurekli et al. (2001). With some modifications, analysis of the plant hormones was according to the methods reported by Izumi et al. (1988) and by Nefedieva (2003). Extracts

were dissolved in a small volume of methanol and used for HPLC analysis. Samples (20 µL) were injected to a reversed-phase LC18 column (250 × 4.6 mm, 5 µ) (Supelco) connected to an HPLC pump (Cecil 1100, Cambridge, UK), and the column was eluted with a linear gradient using 20–80% methanol in 1% (w/v) aqueous acetic acid at a flow rate of 1.2 mL min<sup>-1</sup> for ABA, with 20–75% methanol in 0.4% acetic acid at a flow rate of 1.0 mL min<sup>-1</sup> for GA<sub>3</sub> and IAA and with 10% acetonitrile at a flow rate of 2.0 mL min<sup>-1</sup> for cytokinins.

Data from three replicates of all treatments were subjected to analysis of variance before the LSD test. A level of  $p < 0.05$  was considered statistically significant.

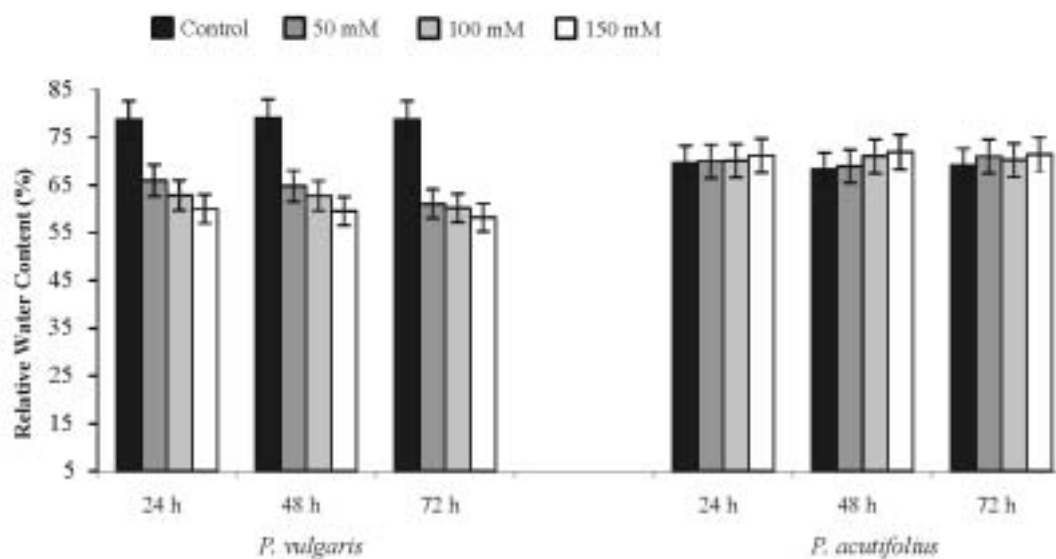
## RESULTS AND DISCUSSION

In this study, two different species of bean plants were compared regarding their hormonal regulation following imposed salt stress in controlled conditions during the early vegetative stage. The results showed that in response to short-term salt stress the common bean (*P. vulgaris*) and tepary bean (*P. acutifolius*) displayed distinct reactions in RWC, soluble protein, ABA, IAA, GA<sub>3</sub> and zeatin content.

### RELATIVE WATER CONTENT (RWC)

In control plants, RWC was higher than 65% in the leaves of *P. vulgaris* and *P. acutifolius* cultivars. RWC in the leaves of *P. vulgaris* decreased with the increase of NaCl concentration (Fig. 1). In *P. vulgaris*, the RWC of leaves decreased significantly at the end of 72 h of salt treatment. Under stress, no significant change occurred in RWC in the leaves of *P. acutifolius* throughout the experimental period. Compared to the control plants, RWC in the leaves of *P. vulgaris* decreased by 61%, 60% and 58% after 72 h at 50 mM, 100 mM and 150 mM NaCl levels, respectively. Only slight variation in RWC was observed in the leaves of *P. acutifolius*.

No significant differences were found in RWC between the control leaves of *P. acutifolius* and *P. vulgaris* throughout the experimental period. However, NaCl treatment had a significant effect on RWC in the leaves of *P. vulgaris*, while no great differences in RWC were found in treated leaves of *P. acutifolius* (Fig. 1). Leaf RWC of NaCl-treated *P. vulgaris* plants was significantly less than that of control plants after 24, 48 and 72 h. Under the impact of short-term salt stress, drought-sensitive



**Fig. 1.** Relative water content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

*P. vulgaris* tended to have lower RWC than the drought-resistant *P. acutifolius* had during the stress period. Similarly, Lazcano-Ferrat and Lovatt (1999) reported significantly decreased leaf and shoot RWC in varieties of *P. vulgaris*, and greater shoot RWC in *P. acutifolius* than in *P. vulgaris* under drought stress. In their work, shoot RWC in *P. acutifolius* decreased significantly when water was withheld for 14 days, while RWC in *P. vulgaris* decreased significantly only 4 days later. Hence, the capacity of *P. acutifolius* to maintain higher leaf and shoot RWC than *P. vulgaris* under water deficit stress may be attributed to its ability to postpone dehydration (Castonguay and Markhart, 1991, 1992). Thus, in our study, *P. acutifolius* was also characterized by better drought tolerance mechanisms and higher tissue water retention capacity than *P. vulgaris*, leading to better growth under short-term salt stress. Parsons and Howe (1984) found lower water potential in *P. acutifolius* than in *P. vulgaris* at a given RWC. This may account for part of the superior resistance of *P. acutifolius*. The greater turgor of *P. acutifolius* due to lower water potential at low RWC values might be caused by several factors including greater tissue elasticity, active accumulation of solutes or greater solute concentration.

#### SOLUBLE PROTEIN

Compared with the controls, treated plants of *P. vulgaris* had significantly decreased protein content

after exposure to 50, 100 and 150 mM NaCl, and the effect was aggravated with time (Fig. 2). However, the soluble protein content in leaves of treated plants of *P. acutifolius* increased significantly over 24 and 48 h and decreased over 72 h at all salinity levels applied (Fig. 2).

Salt stress significantly decreased protein concentrations in leaves of *P. vulgaris* and increased them in *P. acutifolius* except for leaves of *P. acutifolius* after 72 h of treatment (Fig. 2). Our results are in good agreement with the findings of Lazcano-Ferrat and Lowatt (1998), who also found decreased levels of soluble protein content in *P. vulgaris* and increased levels in *P. acutifolius* under water stress. Camacho-Barron and De-Meja (1998) also reported more protein in *P. acutifolius* than in *P. vulgaris* under water stress. Supporting our results, Sibole et al. (1998) also found severely reduced leaf protein content in *P. vulgaris* under short-term salinity. Similarly, in a study of three rice cultivars (*Oryza sativa*) with different levels of drought tolerance, synthesis of several new polypeptides was observed, assumed to have a role in water stress tolerance (Perez, 1996). The negative effect of salinization on protein synthesis in salt-sensitive *P. vulgaris* might be attributable mainly to the osmotic effect rather than a salt-specific effect. Stress proteins, including water deficit proteins, osmotic stress proteins and others are abnormal cell constituents generally absent in non-stressed plants. As discussed later, a slight increase in protein content in *P. acutifolius*

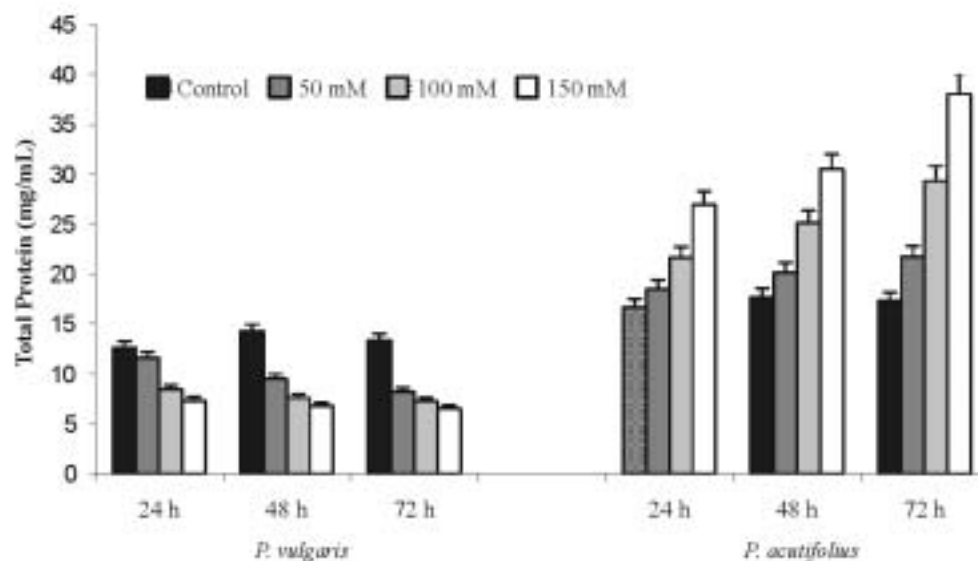


Fig. 2. Total protein content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

was accompanied by an increase in zeatin content. Maab and Klambt (1977) found that kinetin increased protein synthesis by an average 35% in *Nicotiana tabacum*. Similarly, an increase in protein content due to cytokinins was reported by Paranjothy and Wareing (1971) and Short et al. (1974). Although the modes of action of cytokinins on protein synthesis are totally unknown, they might directly affect protein synthesis as well as RNA synthesis.

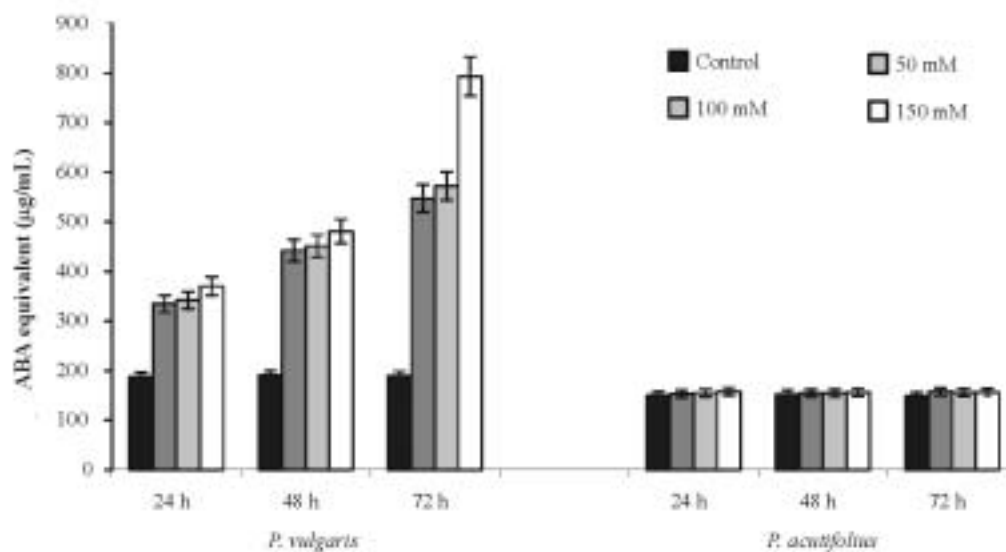
#### ABA, IAA, GIBBERELLIN AND ZEATIN CONTENT

In general our results indicated that the two bean species responded differently to NaCl stress in terms of the hormones studied. NaCl treatments resulted in an increase in IAA, GA<sub>3</sub> and zeatin concentrations in the leaves of *P. acutifolius*. Under salt stress, IAA, GA<sub>3</sub> and zeatin concentrations decreased in the leaves of *P. vulgaris*.

The ABA concentration in the leaves of control plants of *P. acutifolius* did not change significantly after 24, 48 and 72 h (Fig. 3). Similarly, the ABA concentration in the leaves of control plants of *P. vulgaris* also did not change significantly throughout the experiment. However, in leaves of salt-stressed *P. vulgaris* plants, ABA concentrations 24, 48 and 72 h after treatment with all three NaCl levels showed remarkable increases compared to the control leaves.

On the other hand, however, there were no significant differences in ABA concentrations in leaves of *P. acutifolius* plants over 24, 48 and 72 h of 50, 100 and 150 mM NaCl administration. The increase in ABA in *P. vulgaris* under salt stress contrasts with the results for *P. acutifolius*, in which there was no such change.

Munns and Termaat (1986) suggest that leaf growth under short-term salinization is controlled by the water status of the root through a root-derived signal, which in certain plants, such as bean, could be ABA mediated (Montero et al., 1997, 1998). Roeb et al. (1982) found that ABA levels remained high in a salt-sensitive cultivar of soybean after growing 168 h in 75 mM NaCl. We also found a significant increase in ABA content in leaves of NaCl-treated *P. vulgaris* 24, 48 and 72 h after treatment with 50, 100 and 150 mM NaCl, while there were no significant changes in ABA in leaves of *P. acutifolius* under NaCl stress throughout the experiment. The increase in ABA levels obtained in our experiment can be compared with the results in grapevine plants (Downton and Loveys, 1981), maize seedlings (Lechno and Baker, 1986) and a halophyte, *Sueda maritima*, treated with NaCl (Clipson et al., 1988). An increase in ABA concentration in leaves under stress is thought to occur due to de novo synthesis or transport from roots (Cornish and Zeewart, 1985; Zhang et al., 1987). Endogenous leaf ABA concentrations have been found to be significantly correlated with leaf



**Fig. 3.** ABA content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

water status (Kannangara et al., 1982) or the osmotic potential of the root medium (Ribaut and Pilet, 1991). In our work, as already mentioned, we observed that *P. acutifolius* had greater leaf RWC than did *P. vulgaris* under salt stress. Hence, the higher ABA concentration in the leaves of *P. vulgaris* may have been triggered by altered water relations rather than by a specific salt effect, as also reported by Kannangara et al. (1982) in leaves of *Sorghum bicolor* subjected to drought stress. Moreover, a lower ABA concentration in the leaves of the drought-tolerant species *P. acutifolius* than in the drought-sensitive species suggests that *P. acutifolius* may result from RWC obtained water as *P. vulgaris* under osmotic effect of NaCl stress. Thus it probably did not induce ABA synthesis and accumulation in leaves of this plant; however, this may also be genotypic difference. It cannot be ruled out that in these species ABA has a detrimental rather than a beneficial effect at these levels of salinity. Thus the levels of salt-induced ABA might be negatively correlated with the salt tolerance of these *Phaseolus* species. Supporting our results, He and Cramer (1996) also reported that ABA concentrations were lower in the salt-tolerant *Brassica napus* than the salt-sensitive *B. carinata*. They also found that growth inhibition increased in a hyperbolic manner with an increase in endogenous ABA concentration, indicating that ABA in salt-stressed plants may play a role in inhibition of growth. Talanova and Titov

(1994) observed a considerable increase in the ABA level in leaves of cucumber under low and high temperature and salinity.

The IAA concentration in leaves of *P. vulgaris* decreased with the increase in NaCl during the course of the experiment (Fig. 4). There were significant differences ( $p < 0.05$ ) between treated and control plants after 48 and 72 h of treatment with 100 mM and 150 mM NaCl. For *P. acutifolius* no change occurred in the IAA concentration of control leaves, but IAA increased sharply with increasing NaCl concentrations and reached the highest value 72 h after salinity treatments. There were also significant differences between treated (50 mM, 100 mM and 150 mM) and control plants and within all NaCl treatment groups in this species.

The evidence on the role of endogenous auxin in salt stress experiments is sparse and contradictory (Bradford and Hsiao, 1982). Much more information is needed on salt-induced changes in the synthesis and metabolism of auxin. In this study, IAA concentrations in the leaves of the two studied species differed at all doses of NaCl (Fig. 4). IAA content in leaves of *P. acutifolius* was significantly higher ( $p < 0.05$ ) than in *P. vulgaris* under 50 mM, 100 mM and 150 mM NaCl treatments. In leaves of *P. vulgaris*, salt stress decreased IAA content to ~60% at 50 mM and to ~50% at 150 mM after 72 h of treatment. In the leaves of *P. acutifolius*, salt stress increased IAA content about 3.2 to 5.1-fold through the 72 h of NaCl

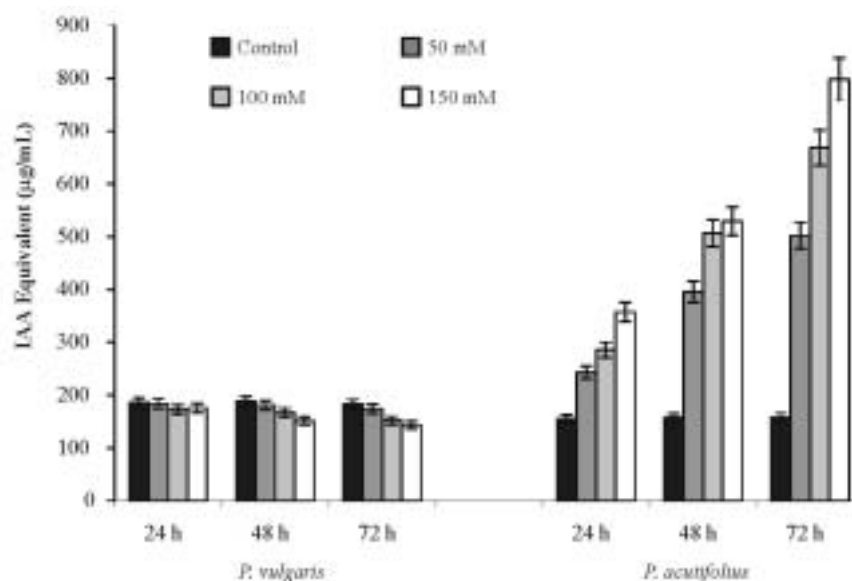
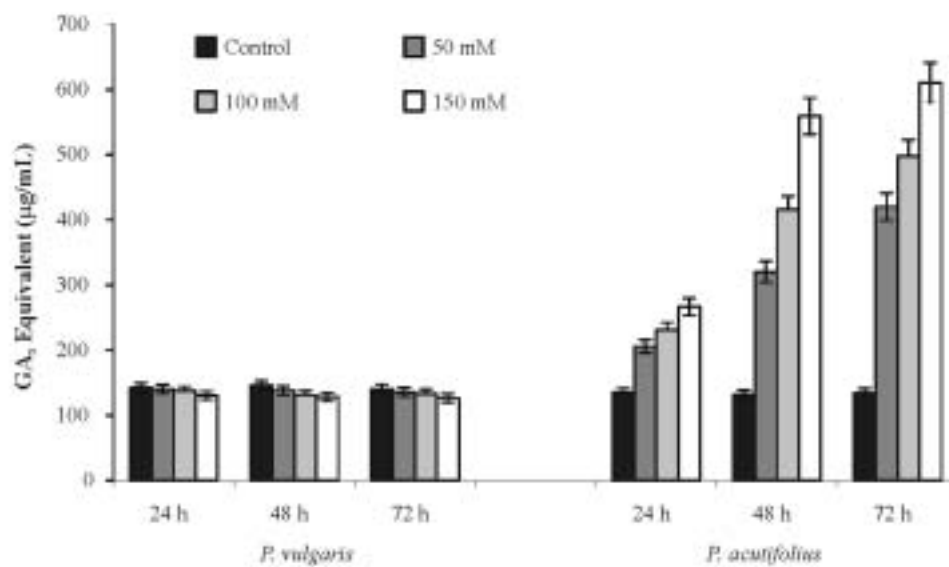


Fig. 4. IAA content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

treatment. This result indicates that the more drought-tolerant and more drought-sensitive species of bean had different mechanisms of control of IAA production. In one of our previous works we observed enhanced IAA levels in the leaves of salt-tolerant wild tomato *Lycopersicon pennellii* than in those of the salt-sensitive cultivated tomato *L. esculentum* under salt stress (Yurekli et al., 2001). In contrast, Naqvi et al. (1986) observed a decrease in IAA content in etiolated *Zea mays* coleoptiles under salinity stress. Similarly, free auxin content was found to decrease with decreasing soil water potential in *Helianthus annuus* (Hartung and Witt, 1968). Kannangara et al. (1982) found no significant relation between auxin content and diurnal changes in leaf water potential or ABA levels in sorghum. Nan et al. (2002) found that in response to water deficit, IAA levels decreased in roots and leaves of wheat in a space experiment. However, confirming our results, Sakurai et al. (1985) observed an increase in the auxin level with decreasing leaf water potential in etiolated squash hypocotyls. Similarly, stress in the form of compacted soil enhanced tissue auxin content in maize root tips (Lechno et al., 1982). Lopez-Carbonell et al. (1994) also recorded increased IAA levels along with ABA in the leaves of *Fatsia japonica* due to the effect of PEG-induced water deficit. Acevedo et al. (1971) observed transitory rapid growth upon osmotic stress release and an increase in tissue extensibility in maize seed-

lings. Veselov et al. (2002) reported that osmotic shock due to PEG treatment resulted in accumulation of IAA in wheat seedlings and suggested that PEG-induced accumulation of IAA in wheat seedlings might be involved in the control of the observed changes in cell wall extensibility, since this hormone is known to increase this parameter (Cleland 1981; Nakano et al., 1981). In our study, enhanced levels of IAA in the leaves of drought-tolerant *P. acutifolius* within 72 h of salt stress may increase leaf cell extensibility, which seems to be involved in maintaining growth under conditions of transient reduced hydration due to salt stress, and seems to promote growth after water relations in the leaf are normalized. Supporting this idea, Li et al., (2003) reported that increased levels of IAA due to GA<sub>3</sub> spraying delayed the biosynthesis of lignin and induced more vigorous growth in bayberry (*Myrica rubra*) leaves.

The total GA<sub>3</sub> concentration in leaves of control plants of *P. vulgaris* did not change significantly throughout the experiment (Fig. 5). However, the gibberellin concentrations in salt-treated *P. vulgaris* plants after 24, 48 and 72 h at 100 mM and 150 mM NaCl treatments were significantly ( $p < 0.05$ ) lower than the control leaves. There were significant differences in total GA<sub>3</sub> content between control plants and those treated with 50, 100 and 150 mM NaCl after 48 h in leaves of *P. acutifolius*.



**Fig. 5.** GA<sub>3</sub> content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

The gibberellin content of the leaves of salt-treated plants of *P. acutifolius* increased remarkably 24, 48 and 72 h after treatment with 50, 100 and 150 mM NaCl (Fig. 5). The rise in GA<sub>3</sub> concentration was pronounced in the leaves of *P. acutifolius*, with about 1.5-, 2.4- and 3.1-fold increases at 50 mM, 1.7-, 3.1- and 3.7-fold increases at 100 mM NaCl and 2.0-, 4.2- and 4.5-fold increases at 150 mM NaCl by 24, 48 and 72 h, respectively. These results clearly show that overall gibberellin levels in the leaves of *P. acutifolius* were affected by NaCl stress, a result that coincides with the results on RWC and protein content in the studied species under NaCl treatment.

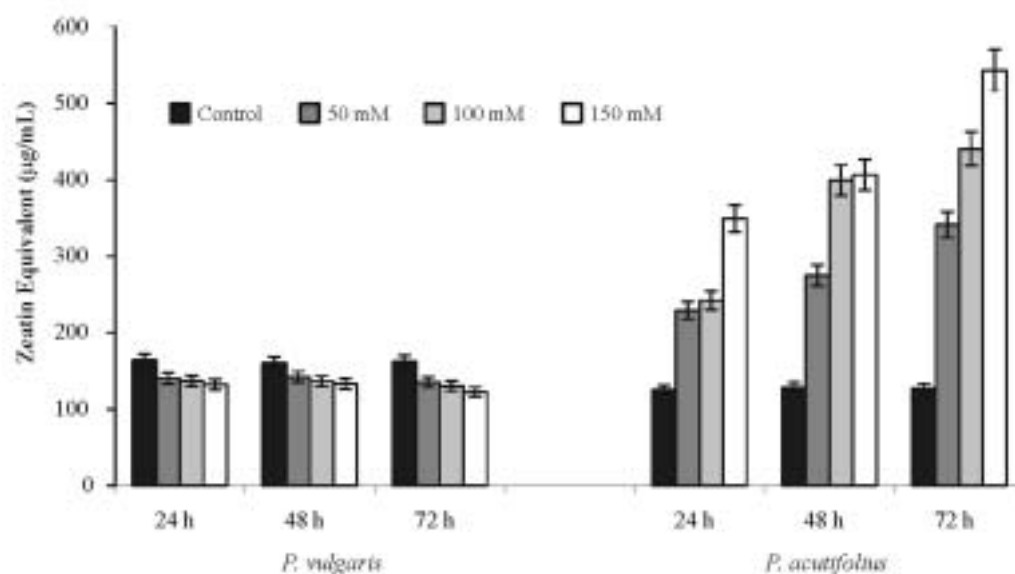
Very little is known about gibberellin levels, particularly under stress conditions. The results differ between types of stress. Aharoni et al. (1977) observed that desiccating excised lettuce leaves showed a rapid decline in gibberellin-like activity; the decline was proportional to the duration and intensity of the stress, and was also closely related to the rise in ABA content. However, we found a remarkable increase in GA<sub>3</sub> content in the leaves of salt-tolerant wild tomato, *L. pennellii* under salt stress (Yurekli et al. 2001). Hubick et al. (1986) found that neither the levels nor the distribution of total free acid gibberellin changed in drought-stressed, aeroponically grown sunflower plants. Exogenous application of gibberellin overcomes the effect of salinity stress on the growth of *Sorghum*

(Amzallag et al., 1992), wheat (Naqvi et al., 1982) and rice seedlings (Prakash and Prathapasenan, 1990). In bayberry, treatment with GA caused an increase in IAA levels of stressed plants, and it was suggested that GA was involved in IAA synthesis or prevention of its degradation (Li et al., 2003). Hence, the higher level of IAA in the leaves of salt-treated *P. acutifolius* might result in increased levels of gibberellin in the leaves.

The zeatin concentration in the leaves of control plants of *P. vulgaris* hardly changed throughout the experiment (Fig. 6). However, zeatin concentrations in leaves of *P. vulgaris* decreased 24, 48 and 72 h after 50 mM, 100 mM and 150 mM NaCl treatments versus the control. In contrast, the zeatin concentrations in leaves of *P. acutifolius* increased rapidly 24, 48 and 72 h after treatment with 50 mM, 100 mM and 150 mM NaCl (Fig. 6). The zeatin concentration increased regularly with the increase in NaCl concentrations, reaching maximum after 72 h at 150 mM NaCl. Significant differences were found between treatments with regard to zeatin concentrations. Zeatin levels of *P. acutifolius* after 72 h showed significant differences between doses ( $p < 0.05$ ). There were also significant differences between treated plants of *P. vulgaris* and *P. acutifolius* after 24, 48 and 72 h at 50 mM, 100 mM and 150 mM NaCl ( $p < 0.05$ ).

Our understanding of the precise role of cytokinins in plant stress response is still limited, and the





**Fig. 6.** Zeatin content in two *Phaseolus* species under salt stress. Results are means  $\pm$  SD of 6 samples  $\times$  3 replicates.

evidence on the effect of stress on cytokinin metabolism in tissues is contradictory. Most of the evidence on the effects of stress on cytokinin metabolism is inferential. In general, xylem exudates and/or leaves of stressed plants exhibit reduced cytokinin activity, and the response is known to be very rapid (Itai and Benzioni, 1976; Wright, 1978; Hubick et al., 1986). In our experiment the concentration of zeatin in the leaves of control plants of *P. vulgaris* remained more or less constant (Fig. 6). In NaCl-treated plants of *P. vulgaris*, the values were lower than those of the control. This might be the result of reduced biosynthesis or enhanced degradation under stress conditions, as was suggested by Vaadia (1976) and Neumann et al. (1990). Cheikh and Jones (1994) found as much as a twofold increase in the activity of cytokinin oxidase in heat-stressed maize kernels as compared with that in non-stressed kernels. Amzallag et al. (1992) reported that salinity triggered an imbalance in phytohormones, including cytokinins. They also observed that exogenous application of cytokinins and gibberellins could reestablish this balance, allowing the plants to grow better at higher NaCl levels. Levels of cytokinins in shoots of drought-stressed sunflower plants were about half those in unstressed shoots (Hubick et al., 1986). Reduced growth rate and reduced shoot:root ratio following salinization of a salt-resistant variety of barley was accompanied by rapid reduction of zeatin content in both roots and shoots (Kuiper et al., 1990).

In contrast, salt-sensitive varieties maintained their growth rates and root:shoot ratios for at least 10 days after exposure to NaCl and displayed no change in the levels of endogenous cytokinins (Kuiper et al., 1990).

On the other hand, *P. acutifolius* responded to all NaCl levels by increasing zeatin concentrations. The increase was about 1.8-, 2.1- and 2.7-fold at 50 mM NaCl, 1.9- 3.1- and 3.5-fold at 100 mM NaCl, and 2.8-, 3.2- and 4.3-fold at 150 mM NaCl after 24, 48 and 72 h, respectively. These results showed that production and/or transport of zeatin were induced in *P. acutifolius* after 72 h NaCl stress. Previously we also observed such an increase in the leaves of the salt-tolerant tomato *L. pennellii* under salt stress. Walker and Dumbroff (1981) recorded a transient rise in zeatin levels for the first 2 days, followed by a depression in the concentration. The reduction in cytokinin activity correlated with reduced growth, and returned to a normal level within 4 days after relief from stress. Similarly, Lopez-Carbonel et al. (1996) reported that zeatin levels in leaves of *Rosmarinus officinalis* increased during the first 3 days of water stress treatment and then slowly decreased during the remaining experimental period. When we consider that exogenous application of kinetin has been used to overcome the effects of different environmental stressors including salinity in many researches, it may be concluded that increased levels of zeatin in leaves of

*P. acutifolius* may enable it to recover faster upon relief from salt stress.

The response of ABA, IAA, and GA<sub>3</sub> and zeatin levels to NaCl stress differed between the drought-resistant and drought-sensitive bean species we studied. Drought-sensitive *P. vulgaris* exhibited higher ABA but lower protein, IAA, total GA<sub>3</sub> and zeatin content with increasing salinity levels, probably due to the osmotic effect of NaCl salinity. In leaves of drought-tolerant *P. acutifolius*, the levels of all the studied hormones except ABA increased within 72 h under NaCl stress.

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