PROLINE RESPONSE OF FABA BEAN (VICIA FABA L.) UNDER SALT STRESS

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Abstract

The response of two faba bean lines (F84 and F85) to the high salinity, 300 and 400 meq of NaCl+CaC12 (v/v) is evaluated by the proline analysis. This marker of the resistance to the environmental stress is analysed in the young leaves, stems and roots aged 24 and 43 days that is to say on the 4th and 23rd days after the salt stress. The amount of proline differs according to the age of the plant, the organ, the line and the treatment. In the stems of F84, an inhibition of proline is revealed on the 4th day of the stress; on 23rd day, this amino acid decreases in the roots stressed. For F85, proline accumulates highly on 4th day in the leaves stressed and on 23rd day, this nitrogen compound is more abundant in all stressed plants.

Key-words: drought-salinity-stress-proline-organ-faba bean-line.

INTRODUCTION

The drought is one fac'or limiting the growth and the development of the plants in the arid zones (Higazy et al., 1995; Belkhodja, 1996). In Algeria, this phenomenon affects 95% of the areas with a rainfull below 400 mm/year (Halitim, 1985). On the other hand, the drought has led to the salinity of the soil characterized by the increasing of the water soluble salts close to the surface (Lieth et al., 1997) and exposed the plants to the continuous stress (Chevery and Robert, 1993). In the medium salts, the plants react to the osmotic adjustment of their cellular middle by accumulating the nitrogen organic compounds such as proline (Sairam and Dube, 1984; Iriguoyen et al., 1992). This amino acid accumulates briskly in many species subjected to the thermic (Paquin, 1986), water (Larher et al., 1993) and slat (Ait Saadi, 1990) stress. We have shown that the faba bean stressed until 200 meq of NaCl + CaC12. 1-1 synthesizes proline at the young stage (Belkhodja and Ait Sadi, 1993; Delkhodja, 1998).

The objective of this work is to investigate the probline ability of the two faba

beaan lines grown under high salt concentrations (300 and 400 meq of NaCl+Cacl21-1).

MATERIALS AND METHODS

Two faba bean lines coded F84 and F85 are used in this experimentation. The seeds disinfected with the sodium hypochlorite and watered with the distilled water, are sown in the sandy pots. On 20th day of post-germination, the young plants are irrigated with the salt solution of NaCl+CaCl2 (V/V) at two concentrations 300 and 400 meq.1-1 of HOAGLAND (1938) nutritious solution and the control with the nutritious solution at 30% of the retention capacity of the sandy substratum.

Two samples are carried out on 4th and 23rd days after the after the salt stress that is to say the plants are 24 and 43 days old. The young leaves, stems and roots are cut separately, weighed and put in a drying room at 80oC during 24 hours. Proline is extracted in each organ with the ethyl alcohol according to the method of AOAC modified by Nguyen and Paquin (1971) and analysed according to the method of Singh et al (1973). The optic density is read at 515 nm on the spectrophotometer type Turner pattern 930. The proline content (µg. mg-1 dry weight) is subjected to statistical test of Student at L.S.D. = 0.05.

RESULTS AND DISCUSSION

1. Variations of the proline contont in the organs of the plants of the F84 and F85 lines.

At first, we present the variation of the proline amount in the young leaves, stems and roots according to the salt treatments and follow by a comparitive study between the lines of proline analysed in each.

Figures 1 and 2 present the proline content in leaves, stems and roots as affected by saltstress. Data (Fig 1 and 2) show that proline amounts determined on 4th and 23rd day of the post stress fluctuate during the development of the plants in the three organs according to the salt treatments.

For F84 line (Fig.1), on 4th day of the stress, in the control treatment proline distributes, in the leaves, the stems and the roots, nearly in the same values. The plants irrigated with the salinity react quickly by an accumulating amino acid particularly in the leaves and the roots; in this case the stems become such as a transit

Fig. 1. Proline content in the young leaves, stems and roots of F84 line analysed on 4th and 23rd day after the salt stress according to concentration of NaCl+CaCl21-1.

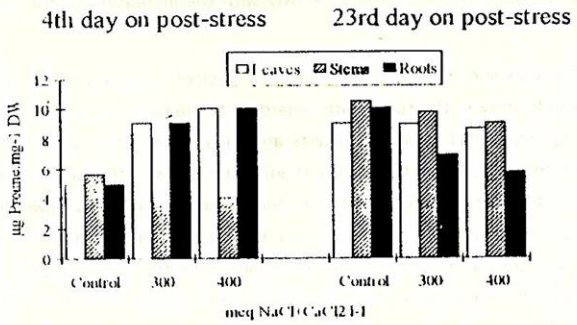
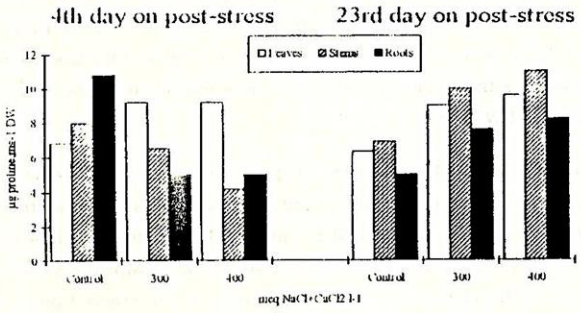


Fig. 2. Proline amount, in the young leaves, stems and roots of F85 line analysed on 4th and 23rd day after the salt stress according to concentration of NaCl+CaCl21-1.



organ with about half of the amount registered in the two others parts. On 23 rd day of the stress, proline increases strongly in all organs of the plants grown without salinity. This nitrogen compound (Proline) accumulates with the same amount in the leaves and the stems either under 300 meq or 400 of $NaCl+CaCl_2$ compared with the control; in the roots, it decreases slowly with the increasing of the concentration of salt.

For F85 line (Fig.2), on 4th day of the stress, in the control proline accumulates significantly in the roots with regard to the leaves and stems (10.8 against 6.8 and 8 ug. mg -1DW). When the plants are irrigated with the salt solution, amino acid is about twice higher in the leaves than in the roots, either at 300 meq salt treatment or at 400 meq one; besides, in the stems, proline falls down significatly with regard to the control (8 against 4.2 µg. mg $^{-1}$ DW). On 23 rd day of the stress, the leaves and the stems grow richer in proline with the increasing of the salinity, in the roots, this enrichment of proline progresses slowly from the control to the salt treatment.

2. An inter lines comparison of the proline amount according to the organs and the age of the plants.

On 4th day after the stress (Fig. 3), a high accumulation of proline in the leaves of the two lines under 300 or 400 meq of salts is observed. In return, proline falls rapidly in the stems of F84 line and slowly in those of F85 line compared with the control. This nitrogen compound (proline) accumulates in the roots of F84 line with the salinity, and its amount is twice higher than for F85 line; whereas for the plants growing without salinity, proline is more important in the roots of F85 line (10.5 µg.mg $^{-1}$ DW against 5 for F84) .

On 23rd day after the stress: (Fig. 4), the leaves of F84 line accumulate more proline without or under salinity and the amount is appreciably the same in the three conditions. (9 µg, 8.9 µg and 8.2 µg. mg-1 DW); then for F84 line, proline is more important only in the leaves of the plants treated with the salinity at the two concentrations. Besides, proline decreases slowly in the stems from the control to salt treatments for F84 line, the inverse phenomenon appears in the stems of F85. In the roots of the plants irrigated without salinity, amino acid presents half of the value for F85 with regard to F84; on the contrary, it increases in the roots of F85 line stressed either at 300 or 400 meq of NaCl+CaCl2.1-1.

Fig. 3. A comparison of the proline amount between F85 faba bean lines in each organ aged 24 days and on 4th after the salt strees according to the concentration of NaCl+CaC121-1.

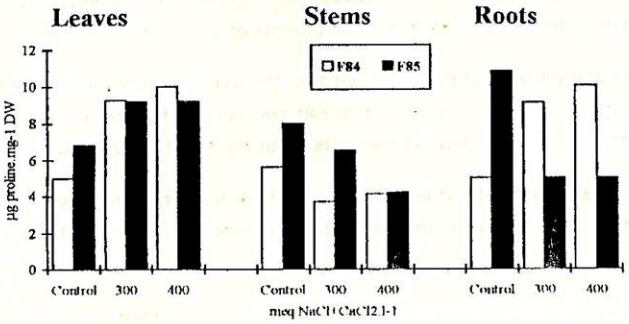
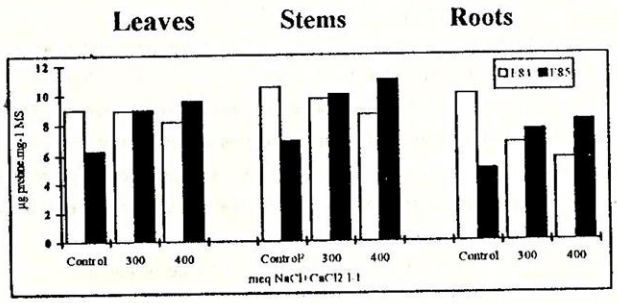


Fig. 4. A comparison of the proline amount between F84 and F85 faba bean lines in each organ aged 43 days and on 4th after the salt strees according to the concentration of NaCl+CaC121-1.



DISCUSSION AND CONCLUSION

Proline analysed in the young organs of the two faba bean lines at 4th and 23rd days of the stress characterizes the variability on the response of the plants cultivated under the high concentrations of salts tested. At 300 and 400 meq of NaCl+CaCl21-1, the plants have experessed a capacity to synthesize proline. Their accumulation fluctuates in each organ in process of time with the salinity.

For the 24days old plants of F84 line, the salinity induces a high foliar a root accumulation of proline under the two salt contrations; for the 43 days old ones, this accumulation slows down in the roots when the salinity increases.

For F85 line, on the stressed plants aged 24 days, the leaves accumulate more proline then this compound increases with the salinity in all organs of the plants aged 43 days.

These results indicates that the young plants stressed until 400 meq of NaCl+Ca Cl_2 1^{-1} produce proline and complete the data already obtained on the same species cultivated until 200 meq of salts (Belkhodja and Ait Saadi,1993). The mechanism of proline accumulation allows to think to a presence of the various sites of resistance of the plants to the stress. These results show that under the salt conditions, the acccumulation of proline varies in the way roots-stems - leaves for the oldest plants (43 days) and for the two lines. On the contrary, this accumulation way appears only for the plants aged 24 days of F85 line; but for F84 line, and for the same age, proline develops perceptibly from the leaves to the roots. Paquin (1986) repeats that proline will be synthesized in the leaves and transfered to the sites of acquiring of the resistance to the aggression. On the other hand, Chen and Wang (1991) bring back that under salt constraints, proline evolves to the leaves and becomes localized in. The data obtained in this experimentation conclude that in the salt mediums, the resistance seems to show itself precociously (on 24 days) in the roots of F84 line and in the leaves of F85 line. This resistance expresses itself later (on 43 days), in the stems and the leaves plants of the two lines. The differences pointed out in the sites presumed of the resistance of each line can come from a precocious water deficit (on 4th day after the stress), particularly in the roots of F84 line, induced by a hard salt stress applied, that is not the case for F85 line in the same period. Also, the high accumulation of proline in all organs about on 23rd day of stress would result from a deficiency of water, the plants were, as a matter of fact, subjected to a continual dryness. Veranjaneyulu and Humari (1989) bring

back that under water stress, proline is less important in the leaves than in the roots by which is transfered the signal of water deficit (Turner, 1986)., signal released by abcissic acid (ABA) (Heino et al., 1990). Rawson et al (1989) assert that at the trong species, the water deficit lead to high synthesis of proline. The accumulation of amino acid remains therefore bound to the availability of water following, according to Irigoyen et al (1992) by a reduction of a cellular water potential.

The accumulation of proline to value the resistance of the plants to the abiotic stress remains discussed. Moftah and Michel (1987) signalize a negative correlation between a tolerance of the plants to the salt or water stress and the accumulation of proline. For Chen and Wang (1991), this accumulation to the acquiring of the tolerancew, expreseed by keeping the turgidity consequently to the osmotic adjustment realized by proline (Turner, 1990). Under salt stress, Weinberg (1987) points out that the proline amount is correlated to the amount of the cations in the leaves, significantly with Na+at *Medicago scutellata* (Refoufi and Larher, 1989). According to Imamulhuq et al (1987), the relation proline - Na+is associated to a water deficit, furthermore, Hayser et al. (1989) suggest that the cationic pool and the accumulation of proline converge to a maintenance of the osmotic balance in the cellu by the hydration of the protoplasm.

The variability in the response of the two faba bean lines through the analysis of proline to value the resistance to the salinity may be the expression of the genes. Bray (1997) has shown that under water stress, some genes, happening in the maintenance of the water potential, code for the pyrroline-5-carboxylase reductase catalyst of the proline biosynthesis (Delauney and Verma, 1993). Besides, Rentch et al (1996) signalize that the gene specifically coded for the transfer of proline (Prot 2) is induced by the water deficit.

The distribution of proline in the organs of the lines stressed remains an insufficient marker. This implicates the coupling of other indicators to clear up the mechanisms of the adaptation to the salinity. The investigation of the characters of the resistance to the salinity by the way of the attended choice by the moleculars markers is an approach to procure the potential begetters for the improvement of the faba bean and to obtain the varieties adapted to the salt soils.

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تأثير الملوحة على انتاج البرولين فى الفول البلدى

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فى تجربة أصص تم فيها تقييم سلالتين من الفول البلدى ((F 84 and F84) على التحمل للملوحة . تم إنبات السلالتين فى الماء المقطر حتى عمر ٢٠ يوم ثم بعدها تم زراعة النباتات فى بيئية رملية وتم ريها بمحلول هوجلاند مع إضافة تركيزين من الأملاح هى ٣٠٠ و ٤٠٠ مليمكافى من كلوريد الكلسيوم (حجم / حجم). تم أخذ العينة النباتية الأولى بعد أربعة أيام من تعرض النباتات للملوحة والثانية بعد ٢٣ يوم. تم فصل الأوراق والسيقان والجذور وتم تجفيفها هواستخلاص البرولين بواسطة كحول الإيثايل تبعا للطرق القياسية وتقديره فى كل منها باستخدام جهاز التقدير اللونى (إسبكتر وفوتومتر) . كميات البرولين المتحصل عليها تم اختيارها إحصائيا بواسطة اختيار الطالب LSD5% Student

وتم عرض النتائج فى شكل أعمدة بيانية. أوضحت النتائج بالنسبة للسلالة F84 أنة بعد أربعة أيام من التعرض للملوحة زادت كمية البرولين فى الأوراق والجذور بينما قلت فى السيقان مقارنة بالكنترول. أما بعد ٢٣٣ يوم فكانت كمية البرولين عالية فى كل الأجزاء النباتية وكانت تقريبا متساوية فى الأوراق والسيقان تحت كل من ال ٣٠٠ و ال ٤٠٠ مليمكلفئ أما فى الجذر فقد تناقصت كميتة عن الكنترول.

أما بالنسبة للسلالة F85 ففى الكنترول وجد أن البرولين تراكم في الجذر بكميات اكثر مما في الأوراق والسيقان عند اليوم الرابع. أما بالنسبة للنباتات تحت تأثير الملوحة فكانت كميات البرولين في الأوراق تقريبا ضعف كميتها في الجذور . ولكن بعد ٢٣ يوم زادت نسبة البرولين في كل الأجزاء عن الكنترول.

من التجربة تبين أن النباتات تحت تأثير الملوحة تزيد مقدرتها علي تخليق مادة البرولين وإن توزيعه تباين داخل الأجزاء المختلفة. هذا التراكم للبرولين في النباتات صغيرة العمر كان أكثر وضوحا. وقد بينت النتائج ان البرولين يتم تخليفة في الجذور ثم ينتقل بعد ذلك للأجزاء الأخري حسب شدة المقاومة للسلالة F84 حيث يتركز البرولين بها في الجذور وفي F85 تتركز البرولين في الأوراق. وهذه المقاومة ظهرت بعد ذلك في السيقان والأوراق.

ومن البحث يمكن القول بأن البرولين يعتبر دليل علي المقاومة للملوحة في الفول البلدي.