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Vigna radiata seed germination under salinity

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Abstract

Salinity reduced mung bean (*Vigna radiata* Wilczek) radicle and root elongation, delayed and inhibited hypocotyl elongation and mobilization of reserves from the cotyledons to the embryo axis. Fresh and dry masses and water content of the embryo axes were reduced. Under salinity, a net leakage of K to the media increased with time and increasing NaCl concentrations. Sugars present in the cotyledons of seeds were of primary importance for growth of the embryo axis upto 18 h after sowing whereas breakdown of starch by amylase contributed later, the contribution being delayed and reduced with increasing NaCl concentration. Even when amylase activity in the cotyledons was progressively reduced with increasing NaCl concentration, the increasing contents of soluble sugars in the cotyledons indicated that sugars were not limiting for mung bean seedling growth under salinity.

Additional key words: amylase, cotyledons, embryo axis, imbibition, potassium, seedling growth, sugars.

Introduction

Salinity is well known to delay or reduce germination and inhibit seedling growth, however, detailed studies on the time course of events during germination are generally lacking. Salinity has been reported to inhibit the first phase of germination which begins with hydration of seeds (Uhvits 1946, Prisco and O'Leary 1970). During this imbibition phase, one of the important processes is the quick re-establishment of the membrane integrity which may be indicated by development of K uptake capacity (Cocucci and Cocucci 1977). Further, reduced growth of the seedlings may result from reduced mobilization of reserves from the cotyledons or from their reduced utilization by the embryo axis. Amylase activity in the cotyledons is generally reduced in dicots under salinity which is coupled with increase or decrease in sugars in the cotyledons (Sheoran 1980, Kocacaliskan 1990, Guerrier 1991, Siddiqui and Krishnamoorthy 1995, Muthukumarasamy and Panneerselvam 1997). The present experiment deals with amylase activities and sugar contents at periodic intervals in the cotyledons and net loss of them into the media at appropriate intervals in relation to growth of the mung bean seedlings under salinity.

Materials and methods

The seeds of mung bean (*Vigna radiata* Wilczek) cv. J-45 were surface sterilized. Ten seeds of uniform mass were placed on a filter paper in 9 cm Petri dishes containing 10 cm^3 of distilled water or 60, 120 or 180 mM NaCl. The Petri dishes care kept in a humidity chamber at a temperature of 30 ± 1 °C in dark. The samples for measurement of radicle/root and hypocotyl length and fresh and dry masses of the embryo axis and cotyledons were taken after 9, 12, 18, 24, 30, 36, 42 and 48 h after sowing.

Potassium contents of the media were determined by flame photometry hourly upto 6 h after sowing for determining its leakage and also at 12, 18, 30 and 42 h after sowing in order to study the re-uptake or further leakage. The total soluble sugars in the aqueous ethanol extracts and amylase activity in the crude extract of the cotyledons in 0.2 M Tris-HCl buffer (pH 7.6) were determined at 6, 18, 30 and 42 h after sowing using antrone (Yemm and Willis 1954) and iodine (Jones and Varner 1967) methods, respectively.

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Results and discussion

Hydration of cotyledons and the embryo axis is the first step in seed germination. The imbibition phase lasted for about 5 h in the present study with *V. radiata* whereas it lasted for about 9 h in *Phaseolus mungo* (Morohashi and Shimkoriyama 1972). Even 180 mM NaCl did not reduce seed hydration to the extent to prevent radicle emergence from the seed coat. The imbibition (Fig. 1*A*) in mung bean was even faster under low salinity (60 mM NaCl) than in distilled water during the peak period of imbibition (2 to 3 h after sowing). As the initial expansion of the embryo axis mainly results from water absorption by the existing cells, the radicle emergence from the seed coat was not delayed by 60 mM NaCl. The radicle emerged from 50 % or more of the seeds by 5 h upto 60 mM, by 6 h with 120 mM and by 7 h with 180 mM NaCl. The data for water content of the seedlings at 9 h after sowing clearly support these observations. Even if the radicle emergence may not depend upon the absorption of water alone, the activation of enzymes or their *de novo* synthesis, which depends upon a certain degree of hydration (minimum water content for germination varies from 25 - 75 % on dry mass basis for different plant species, Brown 1965), would also be delayed by the salinity at or above 120 mM (Figs. 2*E*,*F*) and thus leads to the observed effects on radicle emergence.

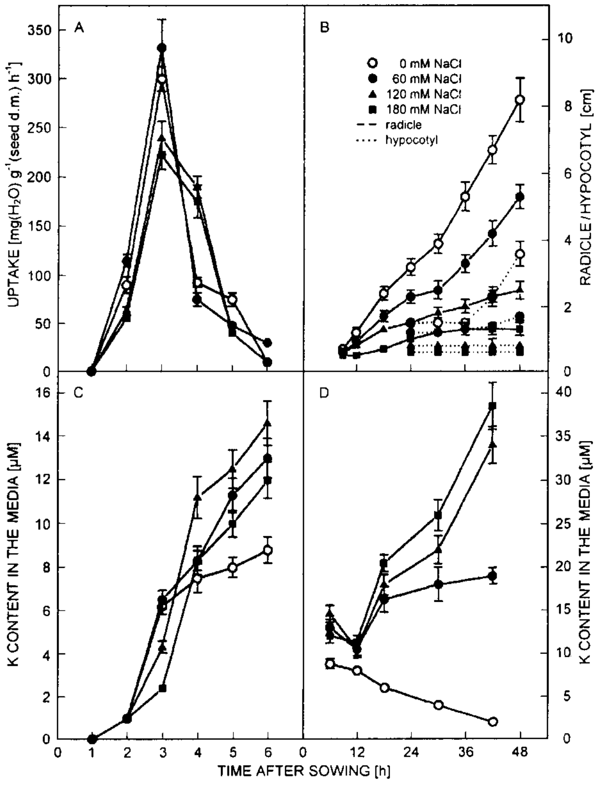

Fig. 1. Effect of salinity on water uptake by the seeds (*A*), radicle and hypocotyl length (*B*) and K content in the media (*C*, *D*).

With the initiation of imbibition potassium is released from the germinating seeds to the media (Simon and Raja Harun 1972). The highest rate of imbibition was observed between 2 and 3 h after sowing under all conditions and the rates of K^+ loss to the media were also maximum upto 60 mM NaCl during this period, but at 120 mM and higher NaCl concentrations, maximum rates were observed between 3 and 4 h (Fig. 1*B*). Further, the net loss of K^+, which continued to take place upto 6 h under all conditions was higher under salinity probably because of a slower re-uptake of K^+ by the embryo axis and cotyledons. A decreased or unchanged K^+ content in the media at 12 h over that at 6 h (Fig. 1*C*) indicated that the rates of uptake were generally faster than the loss of K^+ during this period. A net uptake of K^+ from the media continued to take place under non saline conditions only whereas a net loss of K^+ from the cotyledons/embryo axis continued till the end of the experiment (48 h). Its magnitude increased with increase in NaCl concentration of the media (Fig. 1*C*). As cotyledons are the main source of K^+, it either leaks directly from these or is translocated to the embryo axis and from there it leaks into the media due to altered membrane permeability characteristics or lost in exchange for Na^+. Nassery (1979) has reported K^+ loss from bean roots upon exposure to salinity. Excess NaCl induces changes in bean root structure as well as leakage of ions which were correlated with the alterations in the structure of cell membranes (Cachorro *et al.* 1995). Cocucci and Cocucci (1977) showed that initial K^+ loss was followed by an active K^+ uptake in radish during germination which was inhibited by ABA. Application of ABA promotes Na^+ and inhibits K^+ uptake in bean seedlings (Karmoker and Van Steveninck 1979). Thus the net loss of K^+ to the media observed in the present studies may operate *via* ABA induced increased K^+ release to the media coupled with reduced K^+ uptake due to a low capacity of discrimination for K^+ uptake by K^+/Na^+ system in the presence of NaCl (Benlloch *et al.* 1994).

As stated earlier, radicle elongation was not affected upto 60 mM salinity during first 6 h, however later, the radicle elongation was progressively slowed down with increasing salinity and ceased completely at 180 mM 36 h after sowing (Fig. 1*D*). Hypocotyl, the swollen portion, and root, the rest of descending portion of the radicle, could be distinguished only 18 h after sowing. The hypocotyl elongation was delayed by salinity as it started after 36 h in the control and after 42 h at 60 mM NaCl (Fig. 1*D*); no elongation during the experimental period of 48 h could be observed at and above 120 mM NaCl.

Fresh and dry masses of the embryo axis increased with time and decreased with increasing NaCl concentration (Fig. 2*A*,*B*). The dry mass of embryo axis started to increase by 9 h only, whereas amylase activity was not detected at 6 h and was very low even after 18 h

(Fig. 2D). Between 6 and 18 h, the sugar content of the cotyledons decreased, the decrease being progressively lesser with increasing level of salinity (Fig. 2*C*). As amylase activity had started appearing during this period and increased sharply only after this to contribute significantly to soluble sugars of the cotyledons. The sugars thus present in the cotyledons during this early (upto 18 h) period of growth were primarily responsible for growth of the embryo axis. Simon and Meany (1965) also observed amylase activity to appear in the cotyledons of *Phaseolus* after 37 h whereas dry mass of the embryo axis had started increasing 20 h after sowing. Our results with mung bean also suggest that amylase contribution was further delayed with increasing salinity. Amylase activity sharply increased after 18 h upto 60 mM NaCl and after 30 h with 120 mM and above; the sharpness of this increase progressively decreased with increasing salinity (Fig. 2*D*). Salinity has generally been reported to result in decreased amylase activity in the cotyledons during germination in most of the dicot crops studied (Sheoran 1980 in mung bean, Khatra *et al.* 1985 and Murumkar and Chavan 1987 in chickpea, Kocacaliskan 1990 in common bean, Guerrier 1991 in broad bean, common vetch, lupin and soybean, Siddiqui and Krishnamoorthy 1995 in cowpea, and Muthukumarasamy and Panneerselvam 1997 in radish). On the contrary, amylase activity increased in finger millet (Onkware 1993).

Guardiola and Sutcliffe (1972) have inferred from their results that the factors which affect the growth of the embryo axis affect the transport of reserves from the cotyledons to the embryo axis. Results of De Klark (1986) suggest that embryo axis controls the reserve mobilization in dicot seeds by affecting α amylase activities of the cotyledons. Amylase activity has been observed to be higher in the intact cotyledons than the excised ones under non saline (Karunagaran *et al.* 1992) as well as saline conditions (Guerrier 1991) of different dicot species during germination. Karunagaran *et al.* (1992) also observed total soluble carbohydrate concentrations to be higher in detached cotyledons suggesting that the axis acts as a sink during germination.

In the present studies, in spite of reduced amylase activity (Fig. 2*D*), higher concentrations of sugars (Fig. 2*C*) in the cotyledons under salinity clearly point towards the fact that reduced or complete inhibition of growth of the embryo axis under salinity (Figs 1*D*, 2*A*,*B*) was responsible for reduced or complete inhibition of mobilization of reserve sugars from the cotyledons to the embryo axis rather than hydrolysis of starch to sugar. The point is further supported by the fact that growth of the embryo axis had stopped by 30 h under 180 mM salinity, however, amylase activity appeared to increase even after 30 h and accumulation of sugars was evident in the cotyledons over this period (Fig. 2*C*,*D*). Siddiqui and Krishnamoorthy (1995) observed cv. B9 to increase sugars in the embryo axis without growth improvement under saline conditions indicating that sugars generally do not limit seedling growth in dicots. Absorption of externally applied glucose and its subsequent incorporation into macromolecules of root has been reported to decrease under salinity (Pitman *et al.* 1971, Hasson-Porath and Poljakoff-Mayber 1973). Incorporation of ^{3}H-uridine into RNA and ^{14}C-leucine into proteins has also been observed to be reduced by saline conditions in chickpea embryo axis (Colorado *et al.* 1995).

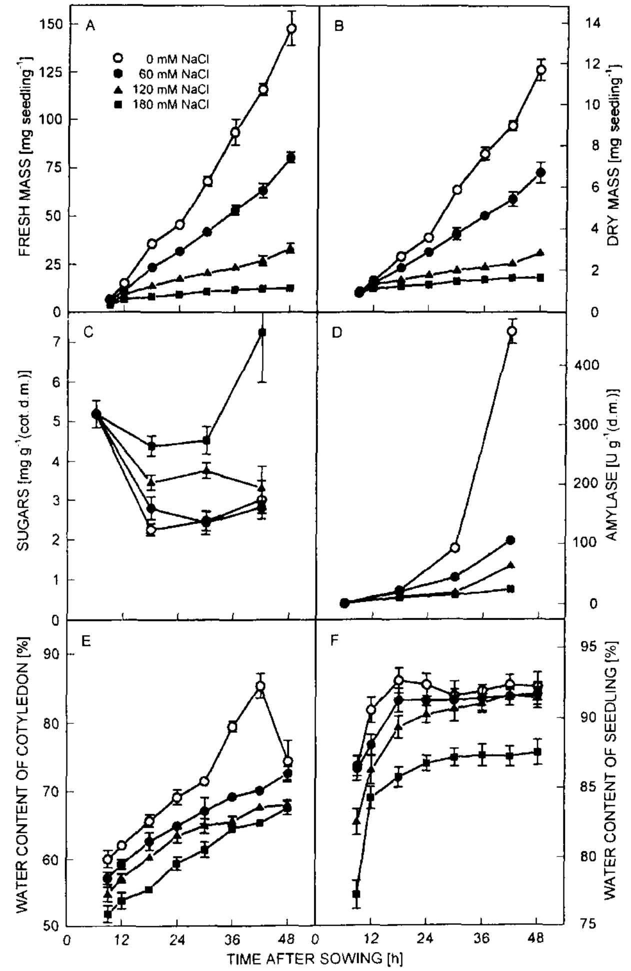

Fig. 2. Effect of salinity on fresh (*A*) and dry (*B*) masses of the seedlings, sugar content (*C*), amylase activity (*D*) and water content (*E*) of the cotyledons and (*F*) of the seedlings.

Water content of the cotyledons and seedlings decreased under salinity (Fig 2*E*,*F*). Water contents of 86 - 88 % should be optimum for mobilization of reserves from the cotyledons to the embryo axis and the attainment of this level was delayed with increased salinity (Fig. 2*E*). Ordin (1960) showed that water stress and decreased internal osmotic potentials in oat coleoptiles were accompanied by reduced incorporation of ^{14}C-glucose into cell wall components. Thus reduced hydration of the embryo axis along with ionic effects of NaCl on the metabolic activities seem to be the factors limiting growth of the embryo axis.

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