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Flowering and male reproductive functions of chickpea (*Cicer arietinum* L.) genotypes as affected by salinity

H.R. DHINGRA and T.M. VARGHESE

Department of Botany, Haryana Agricultural University, Hisar - 125004, India

Abstract

The influence of salinity, given at germination (stage I) or 75 d after sowing (stage II), on flower production and characteristic features of male reproductive structures was studied in three promising genotypes of chickpea (*Cicer arietinum* L. cv. ICCV 88102, H 82-2 and C-235). In ICCV 88102 and H 82-2 salinity 20 meq l^{-1} increased the number of flowers when applied at both stages whereas in C-235 only when applied at the later stage. The salinity also delayed flowering; the higher salinity the greater was delay in flowering. In H 82-2 and C-235 salinity treatment given at stage II (when few flower buds appeared) hastened the flowering. The salinity curtailed pollen production; the reduction being minimum in C-235 and maximum in H 82-2. Germination was not significantly affected in C-235 pollen collected from plants grown under salinity conditions upto 60 meq l^{-1} applied at stage I but the tube elongation was inhibited. The inhibition of tube elongation was greatest in C-235. Salinity treatment administered at stage II did not affect significantly pollen germination excepting C-235 in which a consistent decline with increasing salinity was evident. Salinity stimulated tube growth in ICCV 88102. Na_2SO_4 in the germination medium was more detrimental for both pollen germination and tube growth than NaCl.

Introduction

Salinity is a global problem and like any other environmental hazard, it restrict crop productivity. Adverse effects of salinity are mediated through reproductive fuctions of crop plants. The earlier studies from this laboratory have proved that salinity affects male reproductive functions by limiting pollen production, pollen viability, pollen germination and tube growth (Dhingra and Varghese 1985) or germination alone (Dhingra and Sharma 1992). The vulnerability to salinity is expected to vary with genotype, stage of plant growth at which it is exposed to stress treatment

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(Kaddah and Ghowail 1964, Namuco and O'Toole 1986) and type of salt used (Sheoran and Garg 1983). The effects of salinity treatment given at two stages, *i.e.* germination stage and 75 d after sowing (when few flower buds appeared) on three chickpea genotypes are reported here with reference to flower production and some male reproductive characteristics.

Materials and methods

Seeds of three promising genotypes of chickpea (*Cicer arietinum* L.) namely ICCV 8802, H 82-2 and C-235 were inoculated with appropriate strain of *Rhizobium leguminosarum* and germinated in sand culture. Acid washed sand was filled in the earthenware pots (∅ 30 cm) lined with polyethylene bags having a drainage hole in the centre of sealed end and pots were flushed with N-free nutrient solution (Wilson and Reisenauer 1963). Chloride predominating salinity was created by mixing chloride salts of Na, Ca and Mg and sulphate of Mg to the flushing nutrient solution in the proportion of Na:Ca:Mg 4:1:3 and $Cl:SO_4$ 7:3 on milliequivalent basis. The salinity treatments were given in one set at germination (stage I) and in the second set, 75 d after sowing (DAS) when few flower buds were visible (stage II). The plants were irrigated with nutrient solution at an interval of 15 d. Intermittant water irrigation was also given as and when required. The data on flower production was recorded at 2 d intervals from fifty plants.

Near anthesis, the anthers were cut away from flowers and number of pollen produced per flower was recorded from three replicates of 20 anthers each (Kapoor and Nair 1974). Pollen viability was assessed by 2,3,5 triphenyl tetrazolium chloride (TTC, Hauser and Morrison 1964) and by *in vitro* germination method. Pollen were germinated at 25 ± 1 °C for 4 h on a medium containing 0.5 M sucrose, 0.01 % each of boric and calcium nitrate and 0.7 % agar. Counts for germination and tube length were recorded as described earlier (Dhingra and Varghese 1985).

To study the effect of salts on germination, pollen grains collected from the plants grown under non-saline conditions were inoculated on basal germination medium adjuncted with different levels of NaCl or Na_2SO_4.

Results and discussion

Perusal of data presented in Fig. 1 revealed that production of flowers per plant in three genotypes did not differ significantly. Lower salinity levels (20 to 40 meq l^{-1}) administered at both stages increased the number of flowers significantly in ICCV 88102 and H 82-2. Higher salinities applied at stage I were inhibitory but did not affect flower production in these genotypes if administered at stage II. On the other hand, in C-235 salinity treatment upto 60 meq l^{-1} given at growth stage I did not alter the number of flowers but increased it when applied at stage II. Salinity delayed flowering by 5 - 12 d in ICCV 88102 and by 2 - 6 d in the other two genotypes. Higher the salinity level, the more was the delay. Such a delay in

flowering has been reported for both chickpea cvs. Desi and Kabuli (Datta *et al.* 1987). Salinity treatment given at stage II delayed flowering by 3 d in ICCV 88102 but hastened it by 2 - 4 d in the other two genotypes.

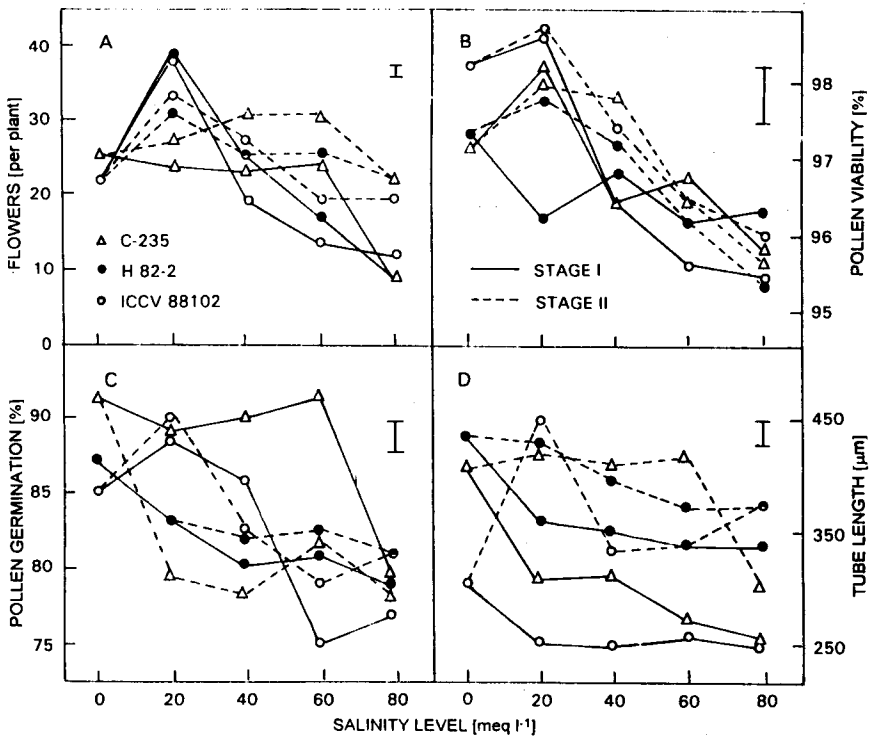

Fig. 1. Effect of salinity applied at germination (stage I) and 75 d after sowing (stage II) on number of flowers per plant (A), pollen viability (B), *in vitro* pollen germination (C) and tube length (D) in chickpea. Vertical bars represent critical difference at 5 % LS.

Flowers of ICCV 88102 produced more pollen (nearly 15 000) than the other two genotypes (nearly 11 000). Flowers of chickpea plants grown under saline conditions produced substantially lower number of pollen, the reduction being least in C-235 and highest in H 82-2 at 80 meq l^{-1} (Table 1). This reduction in numerical production of pollen grains adduces support to the earlier findings from this laboratory in maize (Dhingra 1984), pea (Dhingra and Sharma 1992) and mungbean (Sharma 1992).

Viability of pollen as assessed by TTC staining was about 95 % in all cases and was not significantly affected by salinity treatment given at either growth stages (Fig. 1). These genotypes did not differ much in their *in vitro* germination percentage. But for ICCV 88102, germination remained nearly unchanged in pollen collected from plants raised under saline conditions upto 60 meq l^{-1} at stage I. Higher

salinity, however, was inhibitory (Fig. 1). Pollen of H 82-2 and C-235 plants grown under non-saline conditions produced longer tubes than that of ICCV 88102. Swamy and Khanna (1991) in C-235 reported a germination percentage identical to the present study, but tubes obtained were exceptionally small (10.2 μm only).

Table 1. Effect of salinity given at germination stage on the number of pollen produced per flower in chickpea.

Salinity level [meq l^{-1}]	ICCV 88102	[%] of control	H 82-2	[%] of control	C-235	[%] of control
control	15 712.5 ± 562.1	100	11 581.2 ± 896.6	100	11 838.0 ± 644.9	100
20	13 999.5 ± 1 006.6	89	11 381.2 ± 545.3.	98	9 600.0 ± 167.7	81
40	9 456.2 ± 313.2	60	7 968.7 ± 575.5	69	7 131.2 ± 445.4	60
60	8 486.0 ± 703.4	54	5 587.5 ± 634.7	48	6 517.5 ± 462.8	55
80	6 175.0 ± 261.9	39	3 890.0 ± 459.0	34	6 132.5 ± 298.2	52

Tube length decreased with the increasing level of salinity of the parent plants, the reduction being maximum in C-235 (upto 40 %). In other two genotypes tube lengths of pollen collected from plants grown under 80 meq l^{-1} salinity were reduced to 80 % of their respective controls. Salinity treatment given at stage II did not affect pollen germination except that of C-235 pollen in which a consistent reductiom was evident. A significant enhancement in tube growth of ICCV 88102 pollen collected from plants raised under 20 meq l^{-1} was observed but the values did not differ much from their respective controls in other two genotypes at equivalent salinity. Higher salinity levels were more detrimental to the tube growth of pollen of C-235 than to that of H 82-2.

Addition of 4 mM NaCl to the germination medium improved germination marginally in ICCV 88102 (Fig. 2). Higher concentrations, however, did not alter germination. On the other hand, Na_2SO_4 upto 8 mM did not change germination, but it was inhibitory at higher concentrations. NaCl inhibited germination in the other two genotypes, the inhibition being more in H 82-2 than in C-235. However, Na_2SO_4 was more toxic than NaCl for pollen germination. The tube growth of pollen was stimulated by NaCl (upto 8 mM) in ICCV 88102 and higher concentrations were detrimental. All tested concentrations NaCl inhibited tube growth in other two genotypes. Like germination, Na_2SO_4 barricaded tube growth and maximum inhibition was evident in H 82-2 and least in ICCV 88102 (Fig. 1). This vividly suggest that sulphate ions are more toxic than chloride for both pollen germination and tube growth.

Studies of various workers have revealed that chloride salinity suppressed chlorophyll content, photosynthetic rate (Datta and Sharma 1990), plant growth and economic yield (Lauter and Munns 1986, Manchanda and Sharma 1989, 1990, Manchanda *et al.* 1991) more than that of sulphate salinity. Contrary to this, the present study vividly proves converse to be true for germination and tube growth of

pollen. Studies od Sheoran and Garg (1983) have also shown that Na_2SO_4 is more detrimental than NaCl for seed germination, early seedling growth and dry matter

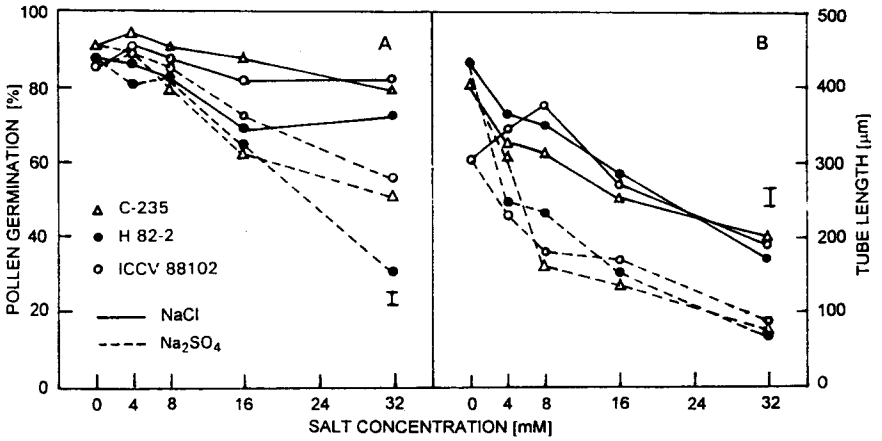

Fig. 2. Effect of NaCl and Na_2SO_4 on *in vitro* pollen germination [%] and tube length [µm] in chickpea. Vertical bars represent critical difference at 5 % L.S.

accumulation in gram. Thus it seems that sulphate ions are more toxic than chloride ions during seed germination and early seedling growth but converse turns true with the advancement of growth. Furthermore, germination of seeds and early growth is comparable to pollen germination and tube growth with respect to their sensitivities to chloride and sulphate ions. This contention is supported by the reported similarities in physiological manifestations of seeds and pollen (Shivanna *et al.* 1991).

References

Datta, K.S., Dayal, J. Goswami, C.L.: Effect of salinity on growth and yield attributes of chickpea - (*Cicer arietinum* L.). - Ann. Biol. **3**: 47- 53, 1987.

Datta, K.S., Sharma, K.D.: Effect of chloride and sulphate types of salinity on characteristics of chlorophyll content, photosynthesis and respiration of chickpea (*Cicer arietinum* L.). - Biol. Plant. **32**: 391-395, 1990.

Dhingra, H.R.: Physiological and biochemical studies on the effect of salt stress on maize (*Zea mays* L.) pollen. - Ph.D. Thesis, Haryana Agricultural University, Hisar 1984.

Dhingra, H.R., Sharma, P.K.: Reproductive performance of pea (*Pisum sativum* L.) under saline conditions. - Indian J. Plant Physiol. **35**: 198-201, 1992.

Dhingra, H.R., Varghese, T.M.: Effect of salt stresss on viability, germination and endogenous level of some metabolites and ions in maize (*Zea mays* L.) pollen. - Ann. Bot. **55**: 415-420, 1985.

Hauser, E.J.P., Morrison, J.H.: The cytochemical reduction of nitroblue tetrazolium as an index of pollen viability. - Amer. J. Bot. **51**: 748-752, 1964.

Kaddah, M.T., Ghowail, S.Z.: Salinity effects on the growth of corn at different stages of development. - Agron. J. **56**: 214-217, 1964.

Kapoor, S., Nair, P.K.K.: Pollen production in some Indian vegetable crops. - Geobios 1: 71-73, 1974.

Lauter, D.J., Munns, D.N.: Salt resistance of chickpea genotypes in solution salinized with NaCl or Na_2SO_4. - Plant Soil 95: 271-279, 1986.

Manchanda, H.R., Sharma, S.K.: Tolerance of chloride and sulphate salinity in chickpea (*Cicer arietinum*). - J. agr. Sci. **113**: 407-410, 1989.

Manchanda, H.R., Sharma, S.K.: Influence of different chloride : sulphate ratios on yield of chickpea (*Cicer arietinum*) at comparable salinity levels. - Indian J. agr. Sci. **60**: 553-555, 1990.

Manchanda, H.R., Sharma, S.K., Mor, R.P.: Relative tolerance of pulses for chloride and sulphate salinity. - Indian J. agr. Sci. **61**: 20-26, 1991.

Namuco, O.S., O'Toole, J.C.: Reproductive stage, water stress and sterility. I. Effect of stress during meiosis. - Crop Sci. **26**: 317-321, 1986.

Sharma, P.K.: Studies on the reproductive behaviour of mungbean (*Vigna radiata* (L.) Wilczek) under saline conditions. - M.Sc. Thesis, Haryana Agricultural University, Hisar 1992.

Sheoran, I.S., Garg, O.P.: Effect of different types of salinities on gram (*Cicer arietinum* L.) during germination. I. Seedling growth and water relations. - Indian J. Plant Physiol. **26**: 363-369, 1983.

Shivanna, K.R., Linskens, H.F., Cresti, M.: Pollen viability and pollen vigor. - Theor. appl. Genet. **81**: 38-42, 1991.

Swami, A.V.S.R., Khanna, V.K.: *In vitro* pollen germination and pollen tube growth in chickpea. - Int. Chickpea Newslett. **24**: 24-25, 1991.

Wilson, D.C., Reisenauer, H.M.: Cobalt requirement of symbiotically grown alfalfa. - Plant Soil **19**: 364-373, 1963.

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