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EFFECT OF SALINITY ON POLLEN VIABILITY OF DIFFERENT CANOLA (*BRASSICA NAPUS* L.) CULTIVARS AS REFLECTED BY THE FORMATION OF FRUITS AND SEEDS

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Abstract

Effect of salinity on pollen viability, germination and yield was investigated on 8 canola cvs. Dunkled, Canola-III, Oscar, Ganyou-5, Ganyou-2, Rainbow, Abasin-95 and Westar. Plants were irrigated with non saline, 0.2% (E.C 2.5 $dS.m^{-1}$). 0.4% (E.C 4.5 $dS.m^{-1}$) and 0.6% (6.5 $dS.m^{-1}$) of sea salt concentrations. Plant growth on vegetative as well as reproductive phases was proportionately inhibited with respect to increasing salinity in irrigation water. Pollen viability and germination was in general adversely affected under saline water irrigation in all cultivars. Two cvs. Oscar and Rainbow exhibited good performance in pollen germination and yield in non saline conditions and show slow reduction in the respective parameters at high salinity level. Hence, these two cultivars could be graded as best cultivars with respect to above mentioned parameters.

Introduction

Salinity refers to the occurrence of various salts in soil or water in concentration that may interfere with the growth of plants. It comprises of chloride, sulfates and bicarbonates of sodium, calcium, magnesium and potassium (Anonymous, 1984). Vegetation occurring in various parts of the world are mainly dependent on quality and quantity of available water than on any other environmental factor. Agricultural production is principally dependent on water but the quantity of good quality water is not sufficient to meet the crop water requirements and vast tracts are lying barren due to non availability of this essential input. Brackish water is an alternative of this problem but its continuos use, without proper management deteriorate soil properties resulting in very low yields (Nath *et al.*, 1981 and Chaudhry *et al.*, 1983).

Brassica napus (Canola) a member of Cruciferae family is covered with more bloom than other species eg., *Brassica compestris* (toria or sarson). Its leaves are thicker and bluish-green, flowers are larger than those of toria, pods are thin and long. In the early stages it spread out on the fruit axis. It is very late in maturity and remains green till about the middle of April. Since it ripens when the season is too hot the out turn of seed is often comparatively low.

Salinity dictated abnormal ontogeny and organogenesis during flowering process, in tomato led to fusion of clustered floral buds, resulting into structures resembling capitulum of Asteraceae (Solovev, 1960). The coalesced fruits were either parthenocarpic or contained few seeds. The seed yield in all propensity is determined by number of flowers produced and percentage of these developing into mature filled fruits. Failure of seed formation under saline conditions follow compatible pollination may be assigned to decrease in fecundity of pollen as a consequence of sterility or inability of pollen to germinate on the stigmatic surface, slow growth or bursting of sperms conduit on its way through stylar tissue to embryo sac or to parent-offspring conflict over resource allocation and sibling rivalry leading to abortion of embryos at young stages. *Salsola* species shows significant differences in the structure and ultrastucture of floral organs and pollen morphology (Toderich *et al.,* 2000). Salinity may induce sterility in the pollen, apparently viable pollens may lack the potentiality to germinate or the pollen tube may even fail to fertilize the egg. Experiments were therefore conducted to find the effect of root zone salinity on pollen viability, germination and yield in different canola cultivars.

Materials and Method

Eight canola cvs. Dunkled, Canola-III, Oscar, Ganyou-5, Ganyou-2, Rainbow, Abasin-95 and Westar were used for this experiment. Clay pots containing approximately 3Kg soil each, lined inside with plastic sheats and having a basal outlet for drainage was used in this experiment. Three seeds of each cultivar were sown in 72 clay pots each filled with non-saline soil and irrigated with tap water. Seedlings were thinned to one per pot after 20 days prior to starting saline water irrigation. Different dilutions of sea salt were used in irrigation water for salinity treatment. Out of 72 pots kept for each cultivar 18 replicates were maintained per treatment eg., control (non-saline), 0.2% (E.C 2.5 $dS.m^{-1}$). 0.4% (E.C 4.5 $dS.m^{-1}$) and 0.6% (6.5 $dS.m^{-1}$) sea salt concentrations. Concentrations of sea salt were gradually increased in irrigation water till it reached to the desired salinity of each treatment. Each pot was irrigated with 1.5L of tap water / salt solution twice a week.

Number of flowers and siliquae were recorded weekly. Seed number and weight per plant was recorded at termination of experiment. Total flower shed per plant was calculated by the difference between total flowers and siliquae per plant and expressed as the percentage of total flowers produced per plant.

Tetrazolium chloride test for pollen viability: Pollen grains were collected from plants raised under different concentration of salt in irrigation water and their viability was observed as outlined by Dafni (1992). Sample of pollen was taken in a drop of 0.5% 2, 3, 5 triphenyl tetrazolium chloride (TTC) in the 15% sucrose solution and covered immediately to exclude oxygen, which can inhibit dye reduction. The slide was placed in a Petri dish lined with wet filter paper and kept at room temperature for two hours. Viability was calculated on percentage basis by counting colored pollen grains out of a total of 100 randomly selected pollen grains in each replicate.

Pollen germination: Pollen grains were collected from plants raised under different concentration of salt in irrigation water and germination was observed as outlined by Dafni (1992). A 15% sucrose solution was prepared in a mixture of 50% H_3BO_3 ($2x10^{-3}$ M) and 50% $Ca(NO_3)_2$ ($6x10^{-3}$ M) by volume. Sample of pollen was taken in a drop of the medium and covered immediately to exclude oxygen. The slide was kept in a Petri dish lined with wet filter paper. The preparation was kept at room temperature for one and a half an hour. Germination based on protuberance of pollen tube was calculated on percentage basis by observing 100 randomly selected pollen grains in each replicate.

Results and Discussion

Total flowers per plant: Reduction could be cumulative effect of various factors such as decline in number of flowers (Bishnoi *et al.*, 1990; Sharma, 1992), faulty development of pollen grain and ovules resulting improper fertilization and denature embryo, reduction in number of siliquae per plant and seeds per pod, production of shrivel seeds etc. (Kumar *et al.*, 1980). Weekly study of number of flowers per plant in different Canola cultivars (Table 1) showed lesser number of flowers as compared to control plants in all cultivars. Two cvs. Oscar and Rainbow showed high number of flowers in treated as well as in control plants as compared to other cultivars. Some cultivars showed only slight reduction and rather some what promotion in flower formation (e.g. Abasin-95) under saline treatment which could grade them more salt tolerant than others but the total number of flower production was comparatively low hence did not qualify them for high yield cultivars.

Statistical analysis showed significant reduction ($p<0.001$) under saline condition (except Abasin-95 which showed non-significant results).A delay by 15-20 days in flower initiation was most pronounced under various salinity regimes in the present study. There was an increase of about 11% in number of flower formed in Canola var. Ganyou-2 at 0.2% salinity level. Increased production of flowers alone does not help in achieving high yield both in terms of number of fruits or seeds (Dhingra & Varghese, 1997).

Total flowers shed in different Canola cultivars grown under different salinity levels (Table 1) exhibited high values in salinity treated plants as compared to their respective control. With reference to number of flowers per plant, number of siliquae per plant and shedding percentage in higher salinity level cultivars are categorized as follows:

Number of flowers per plant: Oscar > Abasin-95 > Canola-III > Dunkled > Rainbow > Ganyou-2 > Westar > Ganyou-5

Number of siliquae per plant: Oscar > Abasin-95 > Canola-III > Dunkled > Ganyou-2 > Rainbow > Westar > Ganyou-5

Flower shedding percentage: Ganyou-5 > Westar > Rainbow > Canola-III > Dunkled > Ganyou-2 > Abasin-95 > Oscar

Toxic effects on flower shedding was evident and proportionate to increase in salinity of rooting medium in all cultivars. However there was cultivar difference on this parameter. Canola cv. Oscar showing least flower shedding was comparatively more tolerant to salinity in this respect.

Pollen viability and germination: Like other abiotic stresses including light, water deficit, temperature, pollutants etc., salinity also affects pollen performance (Sidhu, 1983; Van Ryn *et al.*, 1986; Alami *et al.*, 1988; Harpen *et al.*, 1988). Viability percentages of pollen grain collected from different cultivars of Canola undergoing irrigation with various sea salt dilutions determined vide tetrazolium salt staining were reduced proportionately with increase of salinity in irrigation water (Table 2). Whereas cvs. Oscar, Ganyou-2 and Dunkled were found 1st three best cultivars in this respect under control (non-saline) conditions. When collected from the plants undergoing 0.6% sea salt

Table 1. Effect of irrigation water of different salinity levels on total flower, siliquae and flowers shed per plant in different Canola cultivars.

Treatment	Total flowers per plant	Total siliquae per plant	Total flowers shed per plant	Flower shedding (%)	Total flowers per plant	Total siliquae per plant	Total flowers shed per plant	Flower shedding (%)
	DUNKLED				CANOLA-III			
Control	182.000 a	129.000 a	53.000 a	29	176.666 a	118.666 a	58.000 ab	33
	±10.440	±8.962	±2.081		±5.696	±8.511	±6.928	
0.2% (S.S)	150.333 b	101.333 a	49.000 a	33	138.666 b	88.000 b	50.666 ab	37
EC_{iw} (2.5 dS/m)	±5.238	±4.666	±4.041		±7.264	±5.507	±5.456	
	(-17.399)	(-21.447)	(-7.547)		(-21.509)	(-25.843)	(-12.644)	
0.4% (S.S)	118.000 c	65.333 b	52.666 a	45	130.333 bc	90.666 b	39.666 b	31
EC_{iw} (4.5 dS/m)	±8.144	±7.172	±15.070		±4.841	±3.282	±6.641	
	(-35.165)	(-49.354)	(-0.629)		(-26.226)	(-23.596)	(-31.609)	
0.6% (S.S)	97.666 c	42.333 b	55.333 a	56	116.666 c	47.666 c	69.000 a	59
EC_{iw} (6.5 dS/m)	±6.064	±14.529	±8.569		±6.064	±7.218	±3.055	
	(-46.337)	(-67.183)	(+4.403)		(-33.962)	(-59.831)	(+18.966)	
LSD0.05	25.237	31.138	29.224		19.664	20.987	18.678	
	OSCAR				GANYOU-5			
Control	303.333 a	222.000 a	81.333 a	27	129.333 a	67.666 a	61.666 a	48
	±49.184	±36.774	±12.547		±11.780	±5.174	±8.875	
0.2% (S.S)	246.333 ab	172.666 ab	73.666 a	30	113.333 ab	39.666 b	73.666 a	65
EC_{iw} (2.5 dS/m)	±27.473	±14.075	±16.505		±16.835	±7.838	±10.088	
	(-18.791)	(-22.220)	(-9.426)		(-12.371)	(-41.379)	(+19.459)	
0.4% (S.S)	180.666 bc	89.000 b	91.666 a	51	81.000 bc	28.333 bc	52.666 ab	65
EC_{iw} (4.5 dS/m)	±9.820	±39.715	±33.193		±3.000	±2.027	±3.480	
	(-40.440)	(-59.910)	(+12.705)		(-37.371)	(-58.128)	(-14.595)	
0.6% (S.S)	132.000 c	82.666 b	49.333 a	37	49.666 c	15.333 c	34.333 b	68
EC_{iw} (6.5 dS/m)	±6.658	±2.728	±6.437		±8.293	±2.603	±6.385	
	(-56.484)	(-62.763)	(-39.344)		(-61.598)	(-77.340)	(-44.324)	
LSD0.05	93.878	91.301	64.673		36.461	16.233	24.913	

Table 1. (Cont.d')

Treatment	Total flowers per plant	Total siliquae per plant	Total flowers shed per plant	Flower shedding (%)	Total flowers per plant	Total siliquae per plant	Total flowers shed per plant	Flower shedding (%)
	GANYOU-2				RAINBOW			
Control	136.666 a	88.333 a	48.333 a	35	237.666 a	174.333 a	63.333 ab	26
	±25.653	±5.333	±22.674		±10.170	±12.115	±6.565	
0.2% (S.S)	151.666 a	77.000 ab	74.666 a	49	192.666 b	120.000 b	72.666 a	38
EC_{iw} (2.5 dS/m)	±12.414	±5.567	±8.875		±3.929	±14.502	±10.682	
	(+10.976)	(-12.830)	(+54.483)		(-18.934)	(-31.166)	(+14.737)	
0.4% (S.S)	109.666 ab	62.333 b	47.333 a	43	115.333 c	76.000 c	39.333 b	34
EC_{iw} (4.5 dS/m)	±7.172	±6.691	±9.386		±4.807	±7.211	±6.359	
	(-19.756)	(-29.434)	(-2.069)		(-55.473)	(-56.405)	(-37.895)	
0.6% (S.S)	76.333 b	37.000 c	39.333 a	51	83.333 d	34.333 d	49.000 ab	59
EC_{iw} (6.5 dS/m)	±14.146	±4.163	±11.609		±3.179	±3.527	±2.081	
	(-44.146)	(-58.113)	(-18.621)		(-64.937)	(-80.305)	(-22.632)	
LSD0.05	53.182	17.977	46.572		20.110	33.479	23.175	
	ABASIN-95				WESTAR			
Control	132.666 a	75.333 a	57.333 a	43	175.666 a	119.000 a	56.666 a	32
	±16.292	±7.125	±10.170		±2.603	±8.326	±6.936	
0.2% (S.S)	114.666 a	48.000 a	66.666 a	58	116.000 b	64.000 b	52.000 a	45
EC_{iw} (2.5 dS/m)	±22.017	±11.590	±10.713		±12.503	±4.041	±13.228	
	(-13.568)	(-36.283)	(+16.279)		(-33.966)	(-46.218)	(-8.235)	
0.4% (S.S)	94.666 a	34.000 a	60.666 a	64	111.333 b	59.333 b	52.000 a	47
EC_{iw} (4.5 dS/m)	±11.050	±8.082	±17.704		±14.193	±8.293	±7.505	
	(-28.643)	(-54.867)	(+5.814)		(-36.622)	(-50.140)	(-8.235)	
0.6% (S.S)	127.666 a	76.666 a	51.000 a	40	67.666 c	22.333 c	45.333 a	66
EC_{iw} (6.5 dS/m)	±31.306	±39.934	±9.018		±6.385	±3.666	±3.382	
	(-3.769)	(+1.770)	(-11.047)		(-61.480)	(-81.232)	(-20.000)	
LSD0.05	70.180	20.043	40.371		32.828	21.127	27.810	

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple range test.
Figures in parentheses indicate % promotion (+) and reduction (-) over control.
S.S= Sea salt

Table 2. Irrigation water of different salinity levels showing percentage of tetrazolium viable pollen in different Canola cultivars.

Treatment	DUNKLED	CANOLA-III	OSCAR	GANYOU-5	GANYOU-2	RAINBOW	ABASIN-95	WESTAR
Control	94.666 a	95.000 a	96.666 a	95.333 a	92.333 a	93.000 a	91.666 a	92.000 ab
	±1.763	±3.055	±1.452	±1.763	±2.962	±2.645	±5.364	±6.000
0.2% (S.S)	76.333 b	86.000 ab	88.333 a	93.000 ab	80.666 a	95.000 a	94.666 a	94.666 a
	±4.096	±2.081	±5.783	±1.527	±6.173	±1.732	±0.333	±2.403
	(-19.366)	(-9.470)	(-8.600)	(-2.448)	(-12.635)	(+2.151)	(+3.273)	(+2.899)
0.4% (S.S)	87.666 ab	85.333 ab	97.000 a	85.666 b	91.666 a	86.000 a	98.000 a	78.000 b
	±5.364	±3.929	±1.527	±3.844	±1.452	±4.582	±1.154	±4.932
	(-7.394)	(-10.100)	(+0.345)	(-10.140)	(-0.722)	(-7.527)	(+6.909)	(-15.217)
0.6% (S.S)	75.333 b	76.333 b	85.333 b	84.000 b	86.666 a	86.000 a	70.333 b	83.000 ab
	±6.359	±2.027	±6.359	±3.214	±2.728	±4.041	±8.212	±2.886
	(-20.423)	(-19.640)	(-11.724)	(-11.888)	(-6.137)	(-7.527)	(-23.273)	(-31.025)
LSD0.05	15.392	9.398	14.431	9.013	12.250	11.218	16.114	14.068

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test.
Figures in parentheses indicate % promotion (+) and reduction (-) over control.
S.S= Sea salt

irrigation, Ganyou-2, Rainbow and Oscar occupied 1st three positions. Germination percentage of pollen grains collected from Canola cultivars undergoing irrigation of different sea salt dilutions was also reduced proportional to increasing salinity of irrigation water (Table 3). Abasin-95, Rainbow and Canola-III = Westar were 1st 4 four best cultivars in this respect under non-saline control whereas Dunkled, Canola-III = Abasin-95 and Oscar = Ganyou-5 occupy these positions when collected from plants undergoing 0.6% sea salt irrigation. This shows that even viable pollen often fail to germinate. Non viable pollen grains under saline condition are reflected in failure of fertilization in different cultivars in present investigation. Adverse effect of salinity on germination of pollen viability has been studied by many workers (Ota *et al.*, 1956; Dhingra & Varghese, 1985; Nagpal, 1991; Sharma, 1992; Sureena, 1994). Salinity has been shown causing sterility as well as inhibition in pollen viability (Abdullah *et al.*, 1978). Akbar *et al.*, (1972) are of the opinion that sterility in rice panicles is due to physiological disorders under salt stress. Nagpal (1991) is of opinion that the adverse effects of salinity on pollen viability, germination and tube elongation are primarily of ionic in nature. Viability of pollen in plants growing under saline conditions was found considerably low in maize, field pea and mungbean (Dhingra & varghese, 1985, Dhingra & Sharma, 1992; Sharma, 1992). However there appears some exceptions, as in chickpea, which is otherwise known for its salt sensitivity where viability of pollen grains were found greater than 95% under saline conditions (Dhingra & Varghese, 1993).

Total siliquae, seed number and weight per plant: Number of siliquae in different Canola cultivars (Table 1) depicts reduction in production of siliquae in different Canola cultivars. Plants irrigated with different salinity regimes showed low number of siliquae as compared to control plants. Francois, (1994) found siliquae reduction in Canola grown under saline condition.

Seed number and weight per plant in different Canola cultivars irrigated with different salinity levels (Fig. 1) showed significant ($p<0.001$) reduction in different salinity levels as compared to control. Cv. Oscar and Rainbow showed high seed number per plant as compared to other cultivars in control as well as in treated plants. In Canola-III 0.2% salinity treated plants showed lower seed number/plant as compared to control and 0.4% salinity treatment, while in Abasin-95 0.6% salinity treated plants showed high seed number and weight per plant as compared to other salinity treatment. In Canola-III 0.2%and 0.4% salinity treated plants showed higher seed weight/plant as compared to control. Salinity of rooting medium has been found responsible for reduction in reproductive yield (Singh & Jain, 1982; Datta *et al.*, 1987; Ram *et al.*, 1989; Mangal *et a.*, 1989, 1990; Manchanda & Sharma, 1990; Dhingra & Sharma, 1992; Dhingra *et al.*, 1995). Shereen *et al.*, 2002 also observed reduction in fertility and yield in rice (*Oryza sativa* L.) under salinity which correspond with the findings of others (Khatun & Flowers, 1995; Khatun *et al.*, 1995; Mohiuddin *et al.*, 1998). Yield calculated in terms of seed weight per plant shows that Canola cvs. Rainbow, Oscar and Abasin-95 occupy 1st three positions under non saline control and Abasin-95, Oscar and Ganyou-2= Rainbow occupied 1st three positions under high salinity of rooting medium (0.6% sea salt). In case cultivars are graded according to their reduction in yield at this higher salinity level in comparison with their respective control, Abasin-95, Canola-III and Oscar occupy 1st three positions (showing 44.0, 56.0 and 66.0%respectively). Therefore if tolerance to salinity is taken as a criteria, the later three cultivars occupy 1st three positions and in case

Table 3. Effect of irrigation water of different salinity levels on pollen germination of different Canola cultivars.

Treatment	DUNKLED	CANOLA-III	OSCAR	GANYOU-5	GANYOU-2	RAINBOW	ABASIN-95	WESTAR
Control	42.667 a	43.444 a	42.444 a	40.556 a	42.333 a	45.111 a	47.000 a	43.333 a
	±3.916	±2.849	±2.467	±2.243	±2.333	±1.767	±2.687	±2.421
0.2% (S.S)	43.333 a	45.222 a	43.000 a	40.444 a	41.111 a	42.000 a	35.889 b	39.111 a
	±2.789	±2.905	±2.014	±2.268	±1.419	±1.683	±2.163	±1.904
	(+1.560)	(+4.092)	(+1.310)	(-0.276)	(-2.887)	(-6.896)	(-23.640)	(-9.743)
0.4% (S.S)	44.667 a	37.000 ab	38.778 a	38.333 a	41.556 a	33.556 b	33.444 b	36.667 ab
	±2.744	±2.794	±2.060	±2.432	±2.375	±0.959	±2.467	±3.682
	(+4.687)	(-14.833)	(-8.637)	(-5.481)	(-1.835)	(-25.615)	(-28.843)	(-15.383)
0.6% (S.S)	36.556 a	32.333 b	31.889 b	31.889 b	31.333 b	27.778 c	32.444 b	29.889 b
	±3.037	±3.249	±1.896	±0.920	±2.321	±1.115	±2.280	±1.136
	(-14.322)	(-25.575)	(-24.868)	(-21.370)	(-25.984)	(-38.423)	(-30.273)	(-31.025)
$LSD_{0.05}$	19.943	11.514	11.914	8.071	10.333	1 0.075	12.986	7.235

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test.
Figures in parentheses indicate % promotion (+) and reduction (-) over control.
S.S= Sea salt

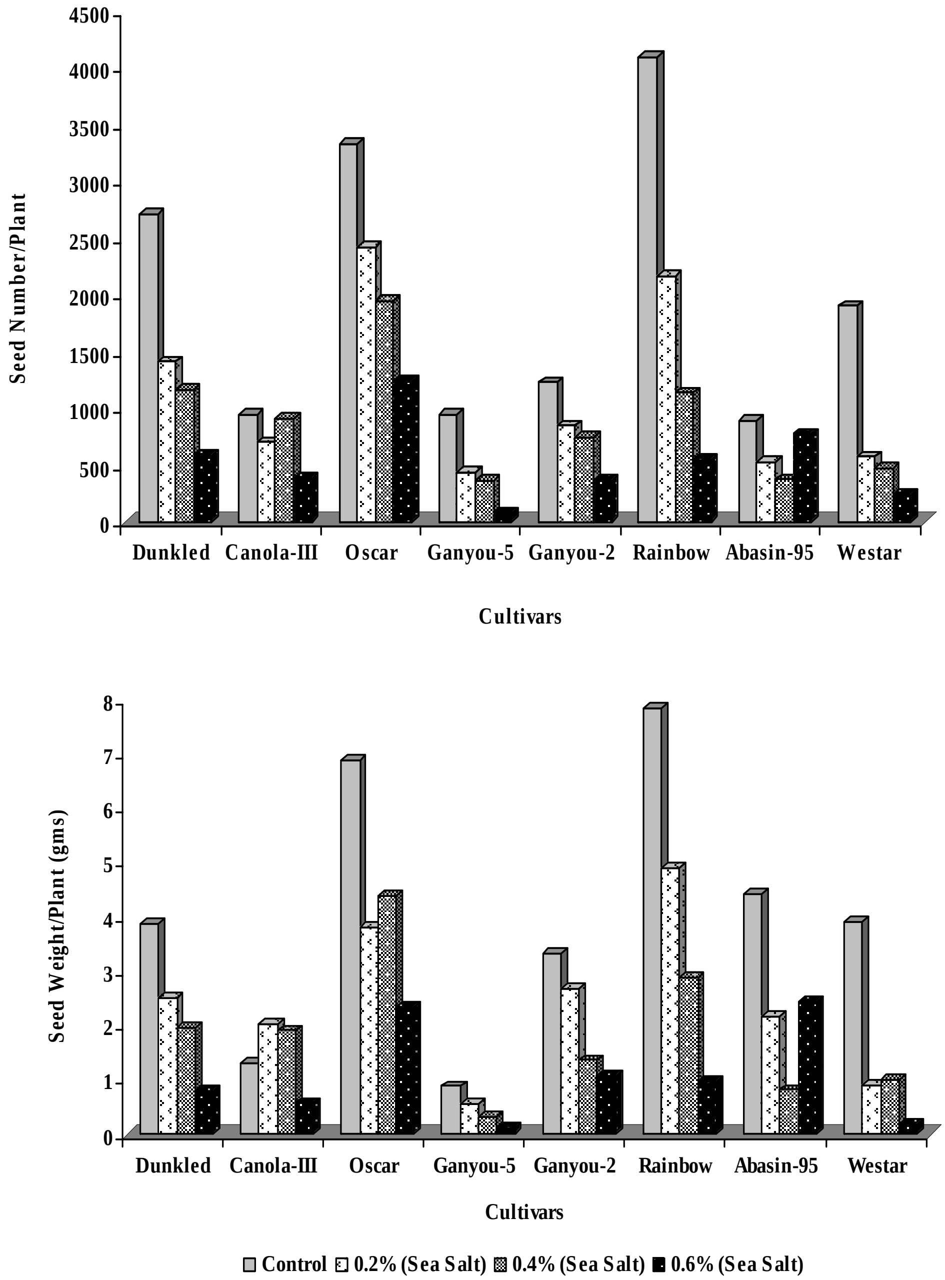

Fig. 1. Effect of different levels of saline water irrigation on seed number and weight per plant of different Canola cultivars.

the yield is concerned the former three cultivars occupy 1st three positions. Cvs. Abasin-95 and Oscar falling with both the categories could be more trustworthy. It is interesting to note that Oscar, Dunkled and Canola-III appear 1st three best cultivars on the basis of vegetative biomass production at 0.6% salinity level. Hence, it appears that certain amount of vegetative growth is essential to supply photosynthates to developing seeds whereas the rest goes wasted in keeping up decorative vegetative format of this plant.

Conclusion

Canola cvs. Oscar and Rainbow could be graded as 1st two best cultivars under non-saline conditions with respect of their yield, in terms of seed weight per plant. Pollen germination and yield was more reduced under irrigation with 0.6% sea salt solution (EC_{iw} 6.8 $dS.m^{-1}$) in all the cultivars. Cv. Abasin-95 showing only 44% reduction in yield at this salinity could also be a good candidate, but its growth vigor was less than that of above-mentioned cultivars. Pollen tube formation leading to fertilization of ovule was in general adversely effected under saline water irrigation. It will be desirable to grow above-mentioned cultivars under field conditions on saline soils/ using saline water irrigation for better yield.

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