significant differences especially Giza 163 which gave the highest values while Yecora Roje recorded the lowest one (Table 5). **Evlagon *et al.* (1990)** found that salinity reduced plant height in maize seedlings. Similar results were also obtained by **Ghulam *et al.* (1997)** who investigated the effect of salinity irrigation on plant height of *Hordum vulgare* cultivars in pot experiment.

Within each cultivar, significant difference was observed between control and salinity treatment for stem diameter (Table 5). As for the overall means, no significant differences were noted between cultivars for this trait.

Regarding the number of leaves/plant, there were no significant differences between means of control and those of salinity stress for each cultivar (Table 5). The overall means of cultivars across the control and treatment showed significant difference between cultivars especially between cultivar G163 which recorded the highest mear compared with Yecora Rogi which gave the lowest mean for this trait.

As for flag leaf area, all cultivars showed no significant differences under salinity stress compared with their respective controls (Table 5). Yecora Roje gave the highest value while Giza 163 gave the lowest one. The means of treatment through all cultivars, gave the highest values than those of control. The overall means indicated marked difference between Yecora Roje (lowest) and Giza 163 (highest) (Table 5). These results agreed with those of **(Munns and Termaat 1986; Munns 1993)** who found that salinity stress led to a reduction in leaf growth rate with associated reduction in leaf area available for photosynthesis.

With respect to number of tillers/plant, both Yecora Roje and Giza163 were not significantly affected by salinity treatment while Sahara 606 suffered significant reduction under salinity when compared with the control (Table 5). On the other hand, the overall means of Yecora Roje significantly surpassed that of each of Sahara 606 and Giza 163 cultivars (Table 5). This was in agreement with **Salam *et al.* (1999)** who found that number of tillers/plant, grains number and one hundred grains weight were more affected by salinity than were plant height, spike length and spiklete numbers.

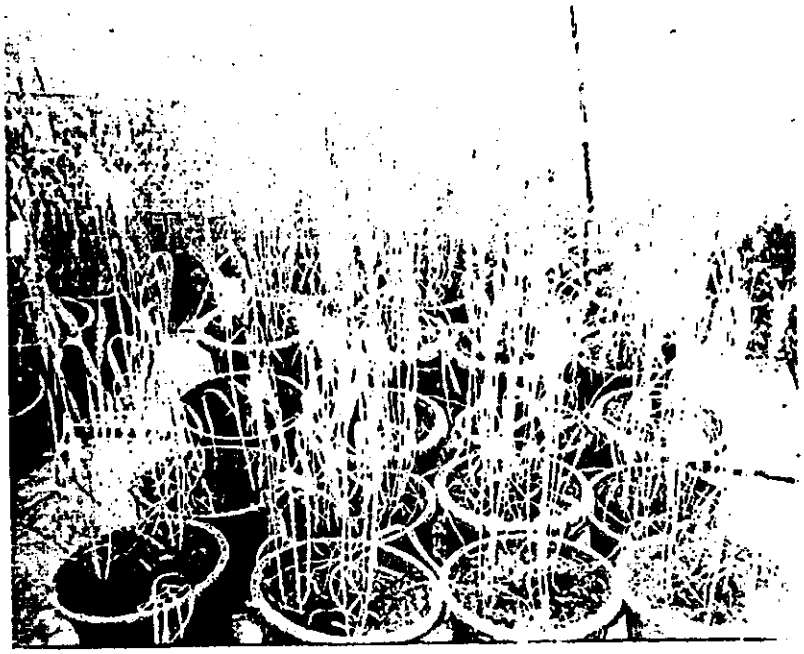

Figure (3): F_2 sensitive group in sand culture.

Table (5): Mean comparisons of some vegetative yield-related traits of three wheat cultivars under control and salinity treatment in a sand culture experiment.

Genotype	Salinity treatment	Plant height	Stem diameter	No. of flag leaves	Flag leaf area	No. of tillers/plant
Yecora Roje	Control	64.88^{C}	0.347^{C}	4.55^{B}	31.83^{B}	4.85^{A}
	Treat.	68.66^{BC}	0.346^{C}	4.45^{B}	33.97^{B}	4.95^{A}
	Mean	66.77^{C}	0.346^{C}	4.50^{C}	32.899^{B}	4.90^{A}
Sahara 606	Control	70.01^{B}	0.436^{A}	4.85^{AB}	36.50^{AB}	4.90^{A}
	Treat.	70.36^{B}	0.420^{A}	4.85^{AB}	37.37^{AB}	4.00^{B}
	Mean	78.28^{B}	0.428^{A}	4.85^{B}	36.92^{AB}	4.45^{B}
Giza 163	Control	81.45^{A}	0.408^{AB}	5.25^{A}	43.604^{A}	3.75^{BC}
	Treat.	81.67^{A}	0.388^{B}	5.20^{A}	37.69^{AB}	3.25^{C}
	Mean	81.56^{A}	0.398^{A}	5.225^{A}	40.647^{A}	3.50^{C}

* Means within columns followed by the same letter(s) are not significantly different by Duncan's New Multiple Range test ($P<0.05$)

Table (6): Mean comparisons of some mature yield-related traits of three wheat cultivars under control and salinity treatment at maturity in a sand culture experiment.

Genotype	Salinity treatment	No. of spike/ plant	Main Spike length	Main Spike weight	No. of spiklets/ main spike	Number of grain/main spike	One hundred grain weight	Grain yield/ plant	Biomass	Harvest index
Yecora Roje	Control	4.75^{A}	11.08^{B}	1.58^{B}	16.85^{B}	32.45^{B}	2.57^{C}	4.37^{B}	10.03^{B}	0.43^{A}
	Treat.	4.75^{A}	10.19^{B}	1.998^{B}	16.60^{B}	37.95^{B}	3.76^{A}	5.25^{AB}	11.78^{B}	0.45^{A}
	Mean	4.75^{A}	10.64^{B}	1.79^{B}	16.73^{B}	35.20^{B}	3.16^{A}	4.30^{A}	10.90^{B}	0.44^{A}
Sahara 606	Control	4.65^{A}	10.40^{B}	2.23^{AB}	21.40^{A}	52.55^{A}	2.71^{C}	6.38^{A}	15.24^{A}	0.42^{AB}
	Treat.	3.80^{B}	10.46^{B}	2.88^{A}	21.15^{A}	56.25^{A}	2.94^{BC}	4.57^{B}	12.03^{B}	0.38^{BC}
	Mean	4.23^{B}	10.43^{B}	2.55^{A}	21.27^{A}	54.40^{A}	2.82^{A}	5.47^{A}	13.63^{A}	0.40^{B}
Giza 163	Control	3.65^{B}	13.22^{A}	2.41^{AB}	21.25^{A}	50.20^{A}	3.48^{AB}	5.13^{AB}	12.54^{B}	0.399^{ABC}
	Treat.	3.25^{B}	11.70^{B}	2.32^{AB}	21.90^{A}	50.45^{A}	2.84^{C}	4.19^{B}	11.37^{B}	0.36^{C}
	Mean	3.45^{C}	12.46^{A}	2.36^{A}	21.58^{A}	50.33^{A}	3.16^{A}	4.66^{A}	11.96^{AB}	0.38^{B}

* Means with columns followed by the same letter(s) are not significantly different by Duncan's New Multiple Range test ($P<0.05$)

At maturity stage, Yecora Roje was the best cultivar in salinity tolerance for the number of spikes/plant while Giza163 was the worst one under salinity stress (Table 6). Within each cultivar, no significant differences were observed between salinity treatment mean and its respective control, with the exception of Sahara 606 which showed a significant reduction. The overall means gave significant differences between cultivars (Table 6). These results agreed with those of **Barakat and El-Haris (1998)** who concluded that number of spikes/plant, grain yield and one hundred grain weight were significantly influenced by the salinity of irrigation water.

Regarding main spike length, no significant differences were observed between the salinity treatment and its respective control within each cultivar except for Giza 163 which exhibited marked reduction below its respective control (Table 6). However, Giza 163 overall mean significantly surpassed those of Yecora Roji and Sahara 606 (Table 6). These results agreed with those of **Barakat and El-Haris (1998)** who found that spikes length in Yecora Roje and grains weight/spike were significantly affected by irrigation water salinity. Also, **Mohamed *et al.* (1998)** reported that increasing salinity levels decreased spike length, grain number / spike and grain yield/ plant of mutant lines of wheat.

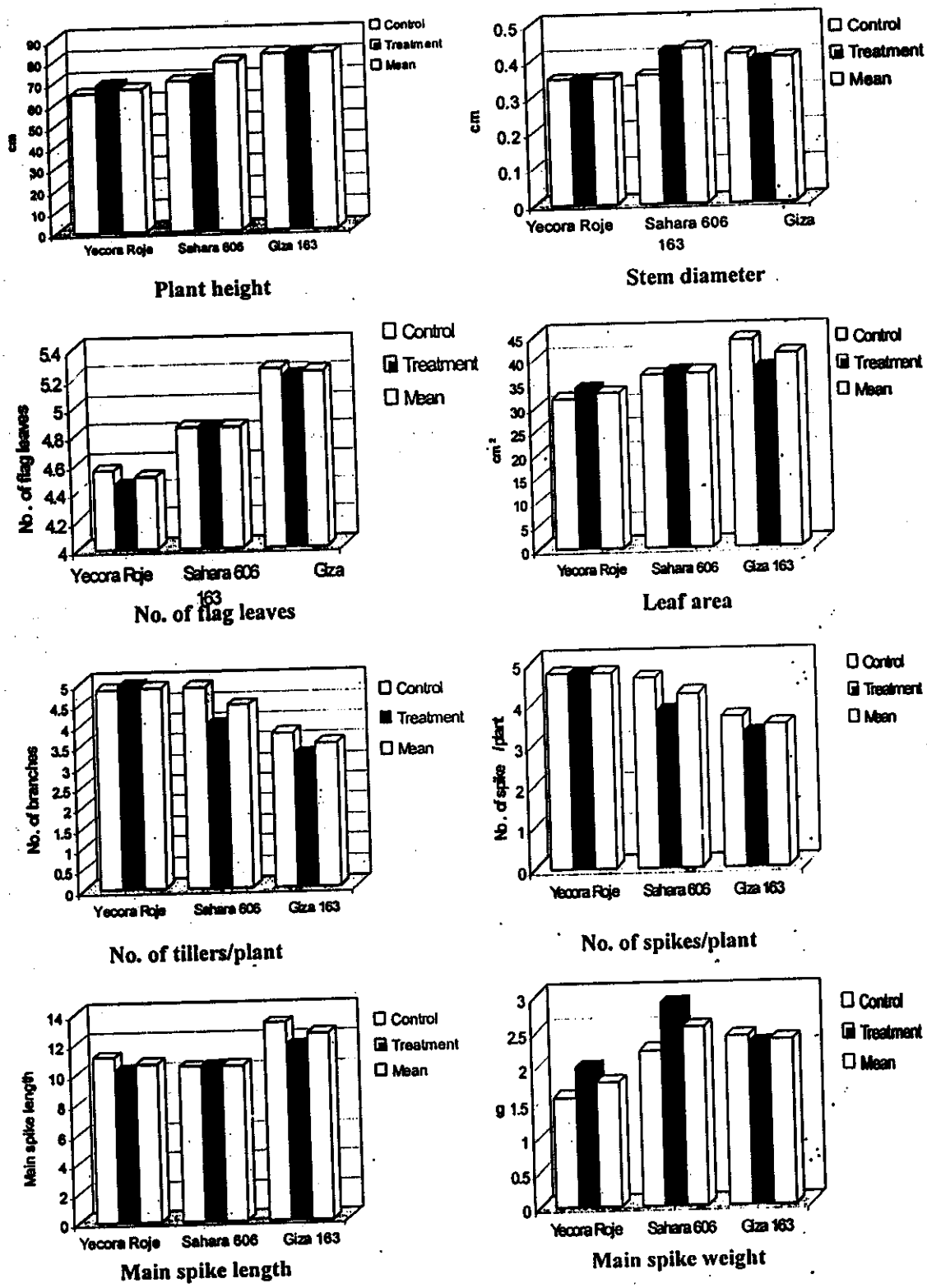

Figure (4): Histograms of means of some stress-related traits of three genotypes of wheat at vegetative and maturity stages.

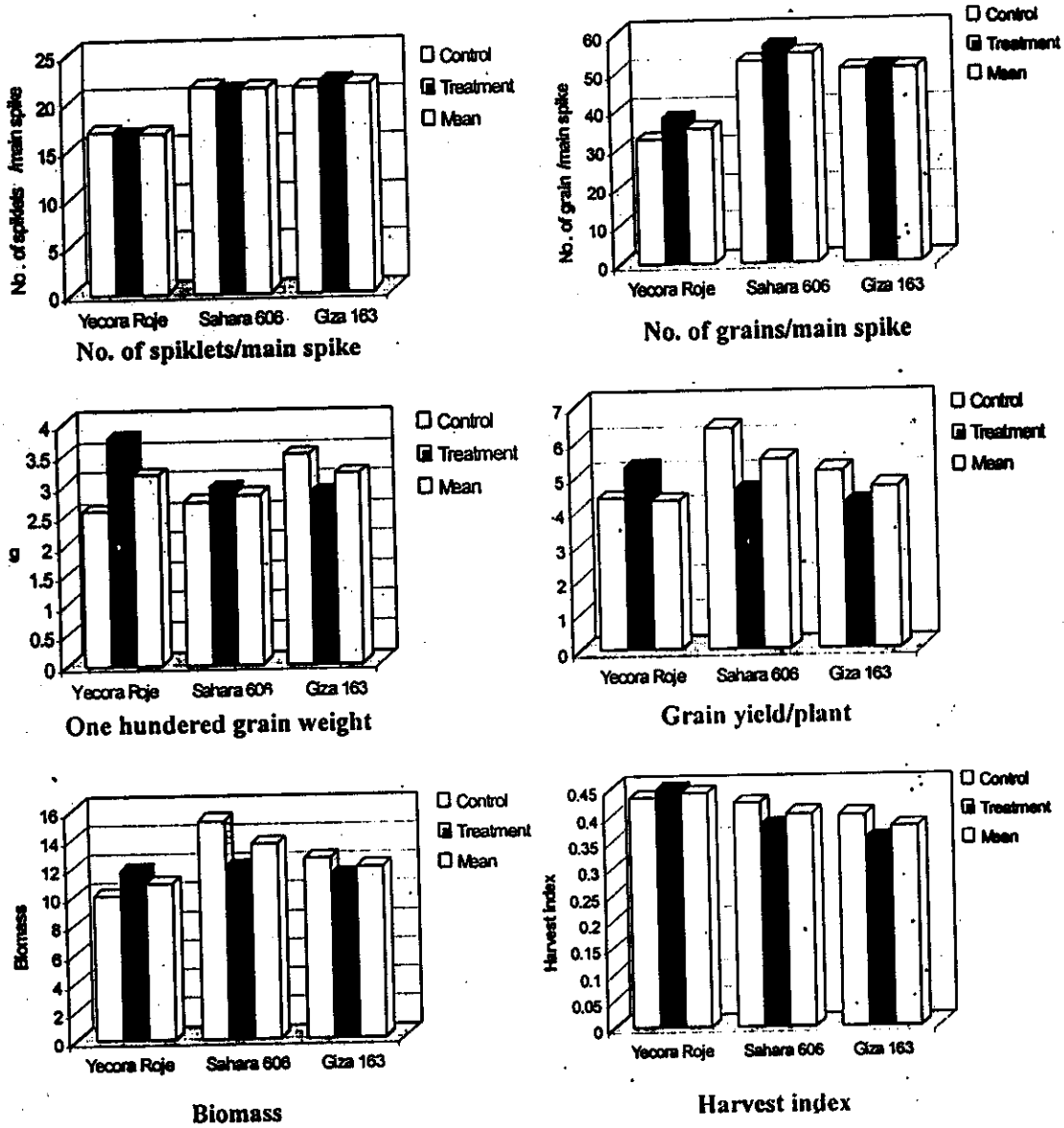

Figure (4): Continued.

As for means of main spike weight, insignificant differences were observed under salt stress as compared with their respective controls, (Table 6). However, the overall means of both Sahara 606 and Giza 163 were significantly higher than Yecora Roje (Table 6). Comparable results were obtained for the number of spikletes/main spike and the number of grains/main spike where the overall means of both Sahara 606 Giza 163 markedly surpass Yecora Roje (Table 6). **Mannus and Rawson (1999)** reported that salinity decreased the formation of spiklete primordia and final spiklete numbers as spikes emergence were reduced.

As for weight of one hundred grains (Table 6), Yecora Roje cultivar performed significantly better under salinity compared with its control, while Sahara 606 seemed not to be significantly affected by salinity treatment. On the other hand, Giza 163 was adversely affected under salinity conditions with substantial reduction in one hundred grains weight trait as compared with its respective control. Nevertheless, the overall means of all three cultivars recorded insignificant differences for this trait (Table 6). Our findings agreed with those of **Abu Khadrah *et al.* (1999)** who found that continuous salinity in water during the growing season significantly reduced plant height, flag leaf arae, total dry matter accumulation, number of spikes/plant, number of grains/spike, weight of grains/spike, 1000 grain weight, grain yield and straw yield of some wheat cultivars.

Regarding grain yield/plant, Yecora Roje cultivar recorded slight increase under salinity compared with the control, while Giza163 recorded slight decrease under salinity treatment (Table 6). However, Sahara suffered marked reduction in grain yield/plant under saline conditions. The overall means of the three cultivars did not differ significantly regarding this trait (Table 6). These results agreed with those of **Hu *et al.* (1997)** who reported that salinity reduced the yield components.

As for total biomass, both Yecora Roje and Giza163 seemed not to be significantly affected under salinity treatment compared with their respective controls (Table 6). On the other hand, substantial reduction in total biomass was observed for Sahara 606 under saline conditions as compared with its control. However, the overall mean of Sahara 606 significantly surpassed the other two cultivars for this trait (Table 6). Our findings are in agreement with those reported by **Kondratjey and Rybkina (1998)** who found that increasing the salinity level led to decrease the K+ in plant organ and increase Na+ concentration. Biomass accumulation decreased and there were changes in plant development compared with control plants. Similar results were also reported by **Sanjatai *et al.* (1999)** who found that the biomass of wheat

cultivars were positively correlated with root dry weight which was decreased under saline and sodic soil conditions.

No significant differences were recorded for each cultivar when comparing its harvest index under saline condition with its respective controls (Table 6). However, Yecora's overall harvest index mean was significantly higher than each of the other two cultivars with laters being insignificantly different from each other (Table 6).

In order to confirm statistically the difference in performance between the two selected cultivars, student t- test was used to compare between twenty individuals of the salt tolerant parent (Yecora Roje) and another twenty individuals of the sensitive parent (Giza 163) for the eleven traits under salt stress (Table 7). Number of tillers / plant, number of spike / plant , main spike length, one hundred grain weight, number of grains / main spike , number. of spiklets / main spike and harvest index showed highly significant differences. However, flag leaf area, main spike weight, grain yield / plant and biomass did not show any significant differences. In general, such results further substantiated the notion that the two chosen parental cultivars indeed represent two contrasting genotypes regarding salt tolerance in this study. Our findings are in agreement with **Midasui *et al.* (1999)** who found that leaf area in sunflower was the most affected trait under salinity treatment, followed by plant height. These results also agreed with those of **Baldini and Vannozzi (1998)** who found that stresses reduced seed yield and harvest index in sunflower. Also, our results agreed with those of **Abdel-Twab *et al.*, (1997)** who investigated the response of seven maize inbred lines to salt stress in order to choose the most tolerant and the most sensitive inbreds. The results of the two maize lines and their F_2 indicated the presence of significant difference between control and treatment. Furthermore, comparable results were reported by **Abdel-Twab *et al.*, (2002)** in their study on drought tolerance in wheat.

F_2 plants (300 individual plants) were classified into groups according to their behavior under salinity conditions in sand culture experiment.

Furthermore, ten F_2 plants representing the most salt tolerant genotypes, and ten F_2 plants representing the most salt sensitive ones were selected on the basis of their performance with respect to eleven traits (Table 8). Comparisons between the means of the two groups regarding each trait indicated marked differences between the two contrasting F_2 genotypes. These results agreed with those of **Abdel-Tawab *et al.*, (1998b)** who evaluated six different traits for fourteen sorghum cultivars to determine the most salt tolerant and the most salt sensitive ones.

Table (7): The t- test of significance for differences between the most tolerant parent and the most sensitive one across eleven characters.

	Independent samples test t - test			
Characters	T	df	P	Significance
No. of tillers/plant	6.6679	38	0.0000	**
No. of spikes /plant	5.7905	38	0.0000	**
Main spike length	-3.9448	38	0.0003	**
Flag leaf arae	-0.8942	38	0.3768	N.S
Main spike weight	-1.250	38	0.2188	N.S
Hundred grain weight	3.4670	38	0.0013	**
No. of grains/main spike	-2.9459	38	0.0055	**
No. of spiklets/main spike	-6.7743	38	0.0000	**
Grain yield / plant	1.6155	38	0.1145	N.S
Biomass	0.3201	38	0.7507	N.S
Harvest index (HI)	4.1068	38	0.0002	**

*p<0.05 **p<0.01 N.S =not significant

Moreover, using t-test for salt stress between the tolerant F_2 plants against the sensitive ones for the same eleven traits (Table 9), showed that all individual plants exhibited significant difference for all the studied traits except number. of tillers / plant and number. of spiklets / main spike. These results agreed with those of **Abdel-Tawab *et al.*, (2002)** who choose two hexaploid wheat cultivars as drought tolerant and drought sensitive genotypes (G 160 and SK 61, respectively) and evaluated them along with their F_1 and F_2 progenies for their relative drought tolerance for some yield- related traits.

Therefore, the selected plants (Table 8) were used to obtain molecular markers associated with salt tolerance by bulked sergeant analysis using RAPD and SSR techniques.

Table (8): Grouping of F_2 plants into two extreme groups; the most tolerant and the most sensitive according to some Yield- related traits.

Plant code number	No. of tillers/ plant	No. of spike/ plant	Main spike length	Flag leaf area	Main spike weight	One hundred grain weight	No. of grain/ main spike	No. of spikletes /main spike	Grain yield/ plant	Biomass	Harvest index
Most sensitive											
1	3	1	11.9	47.88	1.76	2.038	53	24	1.08	5.32	1.08
2	3	3	9.0	52.5	1.66	2.1	50	19	1.76	6.18	0.35
3	3	2	9.2	25.58	1.02	0.886	44	24	0.57	4.22	0.34
4	2	2	10.3	47.25	0.97	0.97	36	23	0.71	3.05	0.28
5	3	3	10.5	37.05	1.85	2.37	51	16	2.63	8.3	0.27
6	3	3	8.0	26.01	1.49	2.78	36	17	1.68	2.99	0.28
7	3	3	8.5	32.03	1.92	2.62	50	15	2.47	5.71	0.23
8	3	2	9.0	50.87	2.3	2.786	56	18	2.63	6.83	0.19
9	3	2	10.5	59.86	1.47	1.57	47	24	1.52	4.78	0.19
10	3	3	10.0	29.7	1.36	2.088	34	17	1.53	6.63	0.11
Mean	**2.9**	**2.4**	**9.69**	**40.87**	**1.58**	**2.0208**	**45.7**	**19.7**	**1.658**	**5.401**	**0.332**
Most tolerant											
11	4	4	12.5	54.34	2.59	3.25	59	16	4.75	10.89	0.44
12	4	3	12.1	52.13	2.59	3.36	58	16	3.15	7.11	0.44
13	3	3	10.9	50.89	2.62	3.10	61	22	4.68	14.53	0.32
14	3	3	10.5	48.31	4.38	3.54	87	19	3.08	6.92	0.45
15	3	3	10.5	46.5	2.98	3.13	63	13	6.1	13.83	0.44
16	3	3	10.3	45	1.98	2.90	50	20	3.67	8.22	0.45
17	3	3	10	43.2	2.55	2.95	64	17	4.74	10.89	0.44
18	3	3	9.5	33.6	2.12	3.47	45	15	2.98	7.1	0.42
19	3	2	9.5	32.25	2.55	3.06	62	19	4.05	9.62	0.42
20	2	2	9.5	29.97	1.9	2.78	50	18	3.18	8.94	0.36
Mean	**3.1**	**2.9**	**10.53**	**43.62**	**2.626**	**3.154**	**59.9**	**17.5**	**4.038**	**9.805**	**0.418**

Table (9): The t- test of significance for differences between the most tolerant F_2 plants and the most sensitive ones across eleven characters. (based on twenty plants of each group)

Characters	Independent samples test t - test T	df	P	Significance
No. of tillers/plant	1.1567	38	0.2546	N.S
No. of spikes /plant	2.3537	38	0.0239	*
Main spike length	2.1990	38	0.0340	*
Flag leaf area	2.1440	38	0.0385	*
Main spike weight	-3.4156	38	0.0015	**
Hundred grain weight	-2.4816	38	0.0176	*
No. of grains/main spike	-4.6792	38	0.0000	**
No. of spiklets/main spike	-0.3876	38	0.7005	N.S
Grain yield / plant	-4.5066	38	0.0001	**
Biomass	-3.7083	38	0.0007	**
Harvest index (HI)	-4.5347	38	0.0001	**

*p<0.05 **p<0.01 N.S =not significant

3. Molecular markers for salinity tolerance via bulked segregant analysis (BSA)

Molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant geneticists. They offer great scop for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular marker linked to that trait, **Mohan *et al.* (1997).**

3.1. RAPD Molecular markers

In this study, we investigate RAPD markers for salinity tolerance in wheat. DNA isolated from the two contrasting cultivars Yecora Roje (ten individual plants) as a salinity tolerant and Giza 163 (ten individual plants) as a salinity sensitive one. Their subsequent F_1 and DNA bulks of tolerant (ten individual plants) and sensitive (ten individual plants) groups in F_2 populations. Their segregation for their response to salinity stress, were tested against six 10-mer random primers. Only, five primers gave polymorphism and developed molecular markers for salinity tolerance. These bands are shown in Figure (5).

These results agreed with those of **(Abdel- Twab *et al.*, 1998)** who investigate nine molecular markers for salt tolerance of sorghum by using RAPD analysis.

The polymorphisms were scored by using eighteen primers, eleven of them detected polymorphism ranging from 1.2 to 22.8%. Six bands were identified on the basis of polymorphic bands of the salt tolerant lines of wheat **(Farook *et al.*, 1994).**

When bulked DNA samples (fifteen tolerant and fifteen sensetive to salt stress) of F_3 populations of two cultivars of wheat were used against seventy four primers. Only, four ones (OPA16, OPM14, OPR14 AND OPZ10) produce polymorphism. Then by using DNA from individual plants, one molecular marker for salt tolerance by only one primer OPZ10 were produced **(Rhaman *et al.*, 1998).**

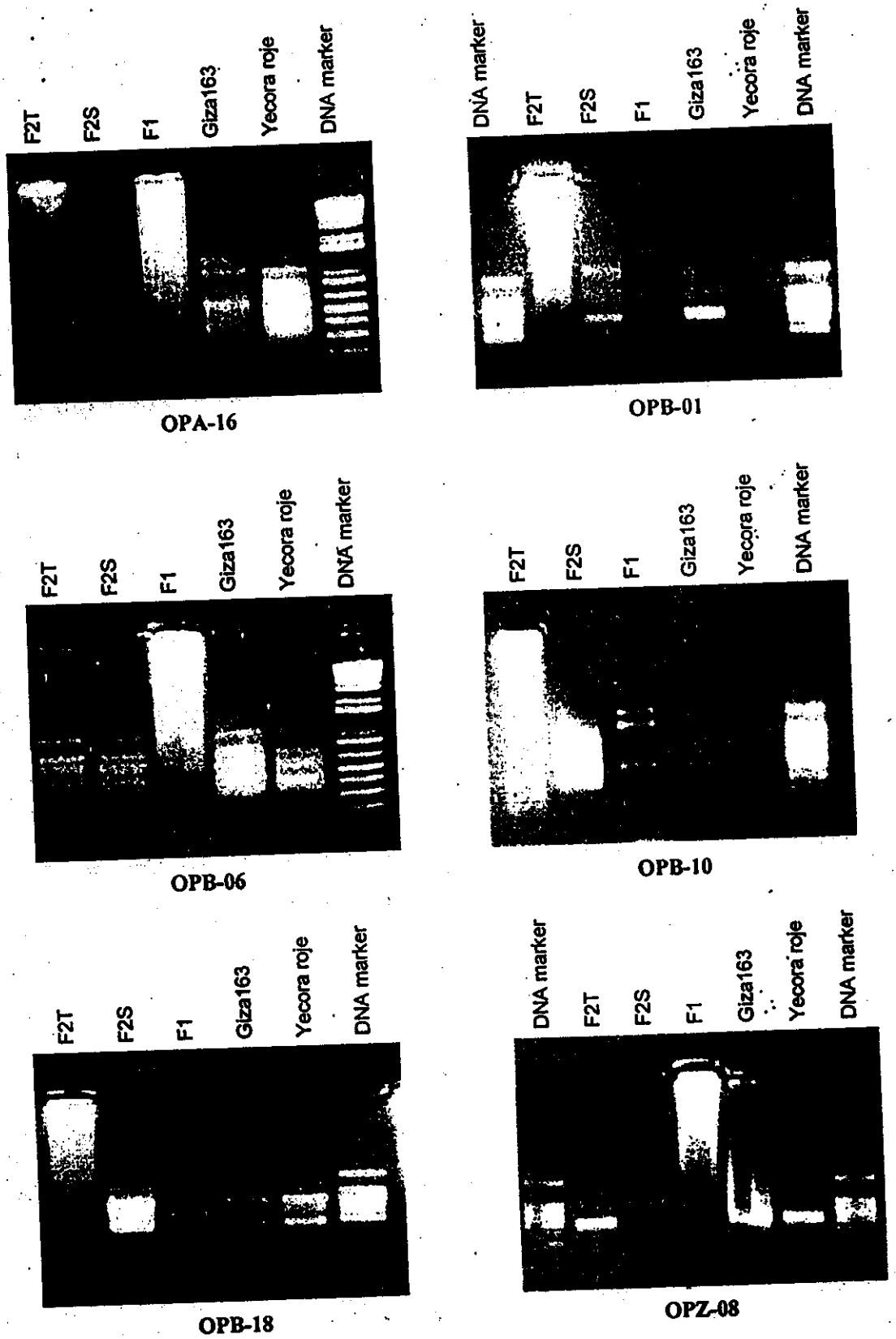

Figure (5): DNA polymorphism using randomly amplified polymorphic DNA with six primers.

Our results are agreed with those of **(Naqvi *et al.*, 1995)** who used the bulked segregant RAPD analysis for a marker linked to single dominant gene (Pi-10t) responseble for blast desease resestance in rice by using 468 random primers. Only, two RAPDs markers linked to the previous gene locus were detected. The linked of these markers were varied using an F_2 segregating populations.

Our results also agreed with **(Abdel-Twab *et al.*, 2002)** who used two contrasting inbred lines of maize,F_1 and F_2 bulked segregant analysis to detect molecular markers for drought, salt and combined effect.

These result confirmed the importance of RAPD markers as a powerful discriminating tool which agreed with **Naqvi *et al.* (1995)** Who suggest that the tightly linked RAPD markers could facilitate early selection of resistance disease locus in rice programs, and with **(Koebner and Martine, 1994; Tyrka *et al.*, 1998)** who detect many successful attempts RAPD markers for the presence of rye chromosomes in wheat background.

Also, these results agreed with **Pal *et al.* (1998)** who used 63 primers for scoring the RAPD markers of the F_2 population obtained from the cross of T. monococcum and *T. boeoticum* and only 38 primers exhibited polymorphism, the size of the amplified products ranged from 0.2 to 4.0 K bp.

3.2. SSR Molecular markers

Simple sequence repeats (SSRs) technique used in this study to obtain molecular markers associated with salinity tolerance by using bulked sergeant analysis. In this study we used fifteen pairs of specific primers developed by Roder et al. (1998) (Table 4). These primers showed different total numbers of bands. Eleven of these primers showed polymorphism and six of them gave seven negative markers, bands associated with salinity sensetive, (appeared in the sensetive parent; G 163; and the sensetive bulck of F_2 and/or not F_1 only). These molecular markers were; one band with molecular size of 703, 479, 415, 201 and 344 bp. which were developed with primers XGMW 33, XGMW 165, XGMW 191, XGMW617 and XGMW 573, respectively. And two

bands with molecular size of 368, 274 bp were exhibeted only with primer XGMW 666.

Forty-five pairs from fif y-three SSR primers pairs produced markers and concluded that this resul: could be compatible with those of expressed sequence taggs (EST) marker in studying functional genomics **(Abdel-Twab *et al.*, 2001a)**.

Also, microsatillite showed a high levels of polymorphism in hexaploid bread wheat more than RFLP and so SSR used in inter-variental breeding applications **(Stepherson *et al.*, 1998)**.

Seven SSRs primers pairs used to differentiate between some winter type durum wheat varieties. The genotypes are all distinguished from each other, with the number of alleles ranging from five to thirteen ones **(Dograr *et al.*, 2000)**.

Microsatellite show a much higher level of polymorphism and information in hexaploid bread wheat than any other marker system **(Plaschke *et al.*, 1995; Roder *et al.*, 1995; Ma *et al.*, 1996; Bryan *et al.*, 1997)**.

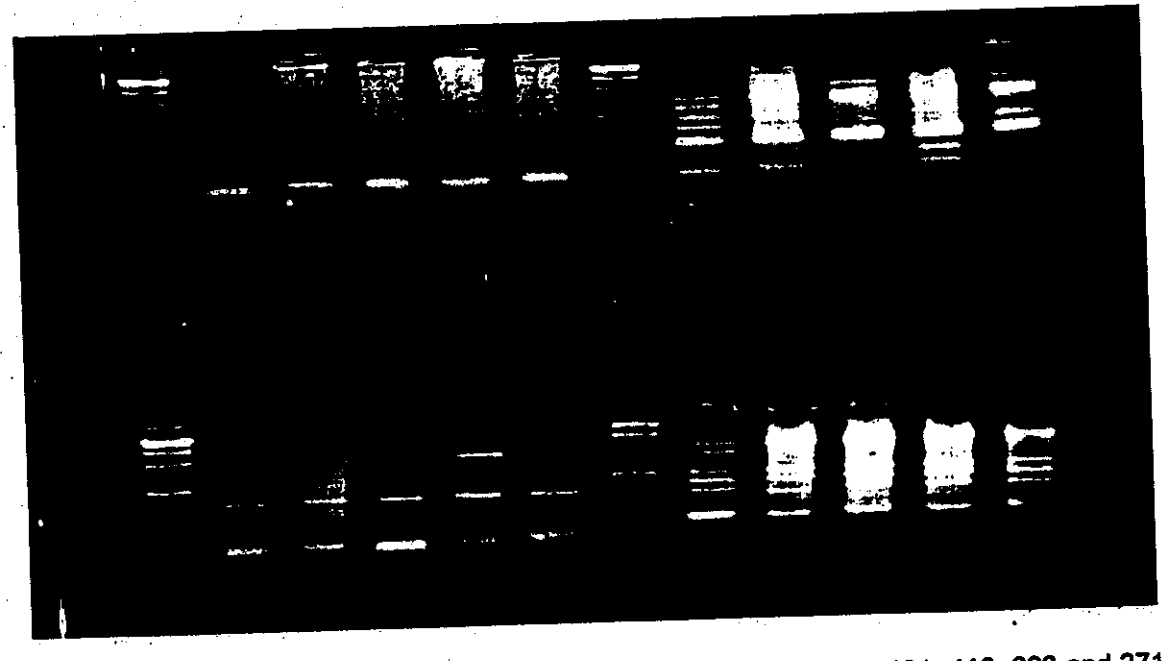

Figure (6): Banding patterns for SSR-based PCR using primer xwmg 121, 410, 666 and 271 for salinity stress by bulked segregant.

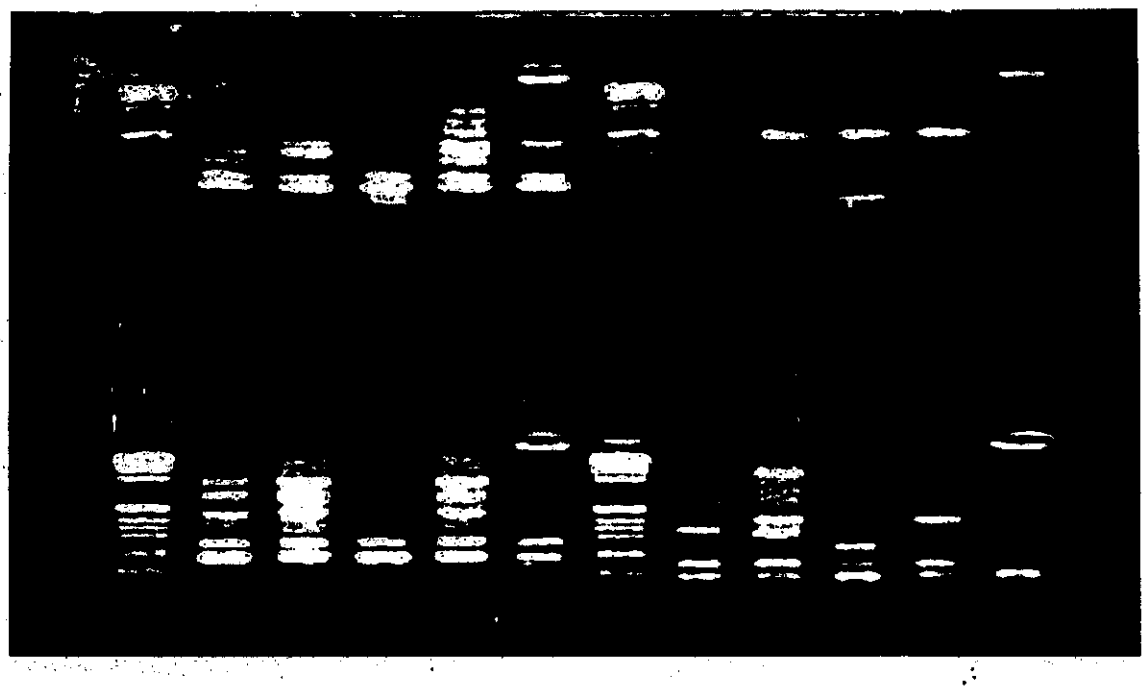

Figure (7): Banding patterns for SSR-based PCR using primer xwmg 573, 33, 165 and 191 for salinity stress by bulked segregant.

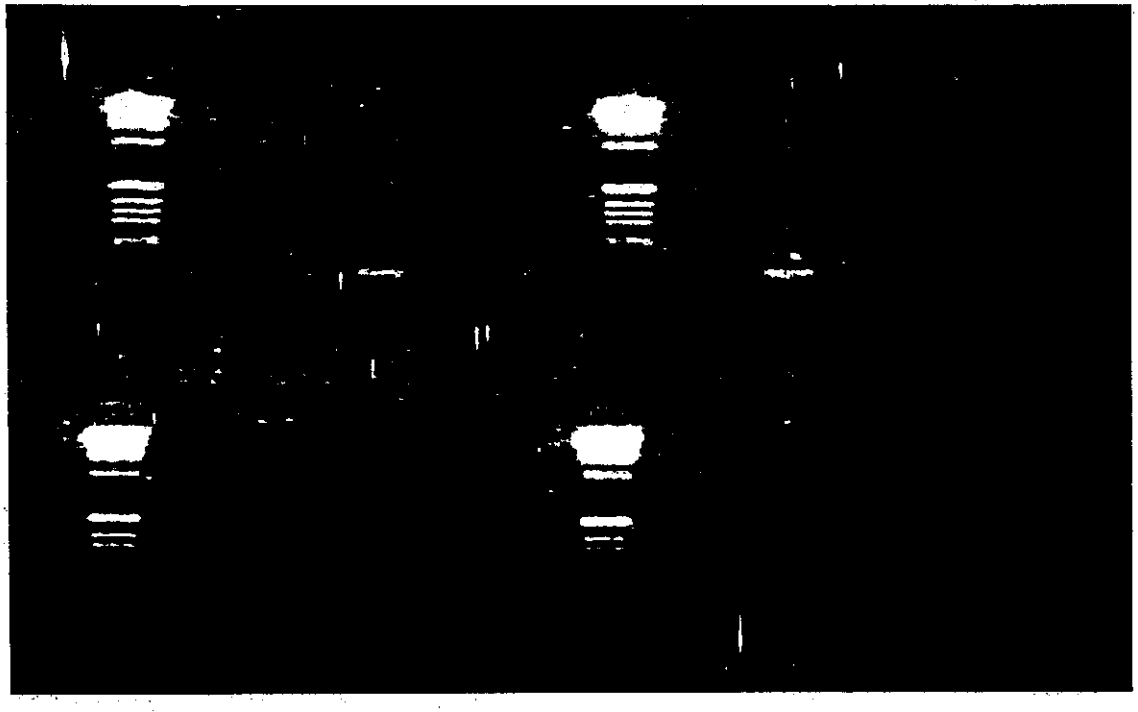

Figure (8): Banding patterns for SSR-based PCR using primer xwmg 608, 617, 112 and 129 for salinity stress by bulked segregant.

The development of microsatellite markers in wheat is extreamly time-consuming and expensive due to large genome size. Only 30% of all primer pairs developed from microsatellaite sequences are functional and suitable for genetic analysis (**Roder *et al.*, 1995; Bryan *et al.*, 1997**). The majority of these markers are chromosome specific.

Finally, wheat microsatillites (WMS) more efficient markers than RAPD markers for studing the population diversity of *Elymus* species because wheat microsatillites (WMS-PCR) detected a much higher level of polymorphism than RAPD analysis (**Genlou *et al.*, 1997**).