

## Apex Development of Three Wheat Cultivars in the Presence of Salinity

M. Kafi<sup>1</sup>

### ABSTRACT

Intra-specific variations in apex development of two salt-tolerant cultivars (Cross Rowshan 11 (CR) and Kharchia-65) and one salt-sensitive cultivar (Ghods) of spring wheat (*Triticum aestivum* L.) grown in sand culture at selected levels of salinity (0, 100, 200, and 300 mol m<sup>-3</sup> NaCl and CaCl<sub>2</sub> in 5:1 molar ratio) were studied. To determine the apex lengths and the number of spikelet primordia in the apex, the main shoot apex was dissected. Results indicated that final spikelet number of wheat cultivars decreased with increasing salinity. Cultivars showed different responses to a particular level of salinity. Duration of spikelet development from double ridge to terminal spikelet, spikelet number and apex length declined in the presence of salinity. Both shorter duration and reduced rate of spikelet initiation were responsible for reduction in spikelet number in salt-treated plants. Cultivar CR showed a better performance during spikelet initiation period and produced relatively more spikelets under saline conditions than the others.

**Keywords:** Apex development, Phenological stages, Salinity, Wheat.

### INTRODUCTION

Salinity is a major constraint on crop production in numerous parts of the world, especially in arid and semiarid regions. The growth of plants may be reduced under salt stress because of osmotic stress due to the lowering of external water potential or to the effects of specific ions on metabolic processes (Munns, 1993). Among crop plants, differences in salt resistance exist not only among different genera and species, but also within species which may on the whole be considered non-salt-tolerant (Epstein and Rains, 1987). Wheat, as the main worldwide staple food, is considered to be relatively salt-tolerant (Bernstein *et al.*, 1974).

A key stage in wheat development following emergence is spikelet initiation. It is a time period between double ridge and terminal spikelet initiation. Terminal spikelet marks the end of initiation of spikelet primordia and thus potential grain sites (Slafer

and Rawson, 1994). Spikelet development is the first step of reproductive stage in wheat plant life cycle and is more sensitive to salinity than later stages (Grieve *et al.*, 1993; Slafer and Rawson, 1994). Grain number in bread wheat and durum wheat was affected mostly by salinity when plants were stressed prior to the booting stage (Maas and Poss, 1989; Christen *et al.*, 1995). Maas and Grieve (1990) demonstrated that salt stress prior to and during spikelet development significantly decreased the yield potential of individual spikes of the sensitive wheat cultivar 'Aldura', but not of the more tolerant wheat cultivar 'Probed'.

Although the effects of some other environmental factors such as photoperiod (Allison and Daynard, 1976; Rahman and Wilson, 1977b; Rahman *et al.*, 1978), irradiance (Allison and Daynard, 1976), temperature (Friend, 1965; Rahman and Wilson, 1977a), nutrient supply (Whingwiri and Kemp, 1980; Frank and Bauer, 1984), and water

<sup>1</sup> Department of Agronomy, Ferdowsi University, P. O. Box: 91775-1163, Mashhad, Islamic Republic of Iran.



stress (Frank *et al.* 1987; Christen *et al.*, 1995) on apex development of wheat have been addressed, there is a little published work on apex development of wheat in the presence of salinity (Taeb *et al.*, 1992). Most of the work has been contributed by the U.S. Salinity Laboratory researchers (Maas and Poss, 1989; Maas and Gerieve, 1990; Grieve *et al.*, 1993), who have reported that spring wheat salt stressed during the vegetative apex stage had a shorter spikelet development duration, which resulted in fewer spikelets per spike. The major effect of salt stress on the timing of shoot primordia initiation is to reduce the duration of the spikelet initiation phase, whereas the period from sowing to flag leaf initiation (last leaf) is unaffected by salinity (Grieve *et al.*, 1993). An acceleration in shoot apex development, as the second major effect, led to reduced spikelet number in each spike, and thus, lower yield potential (Maas and Poss, 1989; Maas and Grieve, 1990; Munns and Rawson, 1999). Similar effects of water stress (drought) on apical development have been reported by Oosterhuis and Cartwright (1983); Frank and Bauer (1984); Frank *et al.* (1987); and Christen *et al.* (1995).

A better understanding of the underlying mechanisms involved in the plant response to salinity is essential to confront this problem. It is suggested that there are associations between particular developmental stage and yield components (Slafer and Rawson, 1994). Since number of spikelet per spike, as a main yield component, is determined in apex development stage of wheat, it is important to be able to manipulate the duration and rate of spikelet initiation. The present work was carried out to study duration of spikelet initiation, spikelet initiation rate, apex elongation rate of three spring wheat cultivars, different in their response to salinity, grown under salt stress. Although wheat is mostly sensitive to environmental temperature during apex development (Slafer and Rawson, 1994), this parameter was kept fixed for all treatments during the experiment in order to study apex development.

## MATERIALS AND METHODS

Experiments were conducted in a growth room with 14 hours day length,  $58\pm 2\%$  relative humidity,  $20/15^\circ\text{C}$  day/night temperature and  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  photon flux density. Three spring wheat cultivars, Cross Rowshan 11 (CR) (Iranian salt-tolerant), Ghods (Iranian salt-sensitive) and Kharchia-65 (standard salt-tolerant) were grown in eight sand tanks in a growth room using sand culture. Four levels of salinity, 0, 100, 200 and  $300 \text{ mol m}^{-3}$  were imposed by adding NaCl and CaCl<sub>2</sub> at 5:1 molar ratio to the modified Hoagland's nutrient solution at two leaf stage (Maas and Poss, 1989). Observation of apex development was started at 20 days after sowing (DAS), based on the results of a preliminary experiment for determining the duration of double ridges (DR) and terminal spikelet (TS), and continued every other day until the terminal spikelet was clearly differentiated.

The plants were given a modified Hoaglands nutrient solution consisting of 2.5 mM Ca (NO<sub>3</sub>)<sub>2</sub>, 3.0 mM KNO<sub>3</sub>, 0.17 mM KH<sub>2</sub>PO<sub>4</sub>, 1.5 mM MgSO<sub>4</sub>, 50 μM Fe as ferric citrate, 23.0 μM H<sub>3</sub>BO<sub>3</sub>, 5.0 μM MnSO<sub>4</sub>, 0.2 μM CuSO<sub>4</sub> and 0.1 μM H<sub>3</sub>MoO<sub>4</sub> (Maas and Greive, 1987) made up in tap water. The nutrient solution was changed every other week. Water lost by evapotranspiration was replenished every day to maintain constant salt and nutrient solution content. The solution pH was maintained between 6-6.5 by adding H<sub>2</sub>SO<sub>4</sub> as required. Salinity was imposed at two leaf growth stage until the end of experiments.

Sampling was done by harvesting two seedlings from each cultivar at different levels of salinity. The main shoot apex was dissected to determine the apex lengths and the number of spikelet primordia present on the apex. When apices had reached the double ridge stage, both the leaf primordium and the spikelet bud could be seen and the number of spikelet buds and lengths of apex were counted using a binocular microscope (Nikon) at a magnification of X40. The dou-

ble unit of leaf primordium and spikelet bud counted as one (Kirby, 1974). Several authors have expressed spikelet duration in number of spikelet per  $^{\circ}\text{C d}^{-1}$ , but since the temperature in growth room was fixed at  $20^{\circ}\text{C}$  in this work, the duration of spikelet initiation, spikelet initiation rate, apex elongation rate were expressed per day. Spikelet initiation rate ( $\beta$ ) and apex elongation rate ( $\alpha$ ) were calculated by dividing the number of spikelets (S) and length of apex (L) (mm) by duration between DR ( $D_1$ ) and TS ( $D_2$ ) (days).

$$\beta = S / (D_2 - D_1)$$

$$\alpha = L / (D_2 - D_1)$$

The experimental design was a split-plot with salinity as main plots and wheat cvs as subplots in a randomized complete block design. Data were subjected to analysis of variance using Minitab Statistic software for Windows version 9.2. Standard error (SE) of significant treatments was calculated by dividing standard deviation by second root of degree of freedom (df) for each particular treatment.

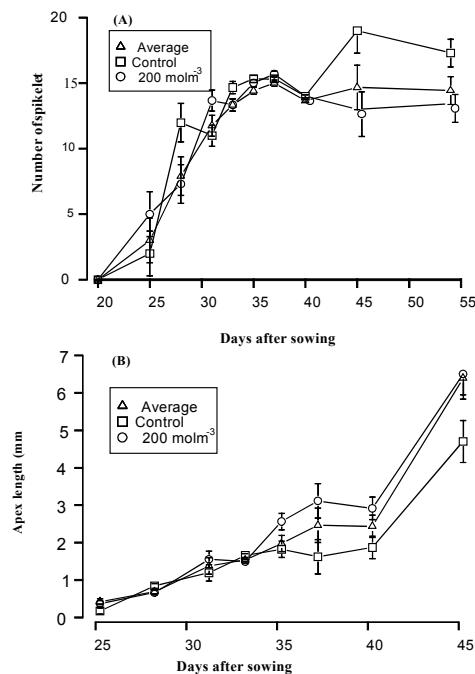
## RESULTS

Figure 1 shows the average spikelet number in non-saline conditions, high level of salinity and average of four levels of salinity from 20 to 55 days after sowing (third leaf to booting stage, the stage that spike located in flag leaf sheath). Average spikelet number per apex and apex length of all cultivars did not show significant changes compared with control until 40 days after sowing (DAS) in the presence of  $200 \text{ mol m}^{-3}$  salinity. No significant difference was observed between salt treatments and control for the rate of spikelet initiation until 40 DAS. Although, at this time terminal spikelet initiation was ceased in salt-affected plants, it continued for five more days in the control plants (figure 1a). These results confirmed that apex development period in salt-treated plants was shorter than that in the control plants. Apex elongation started to increase after

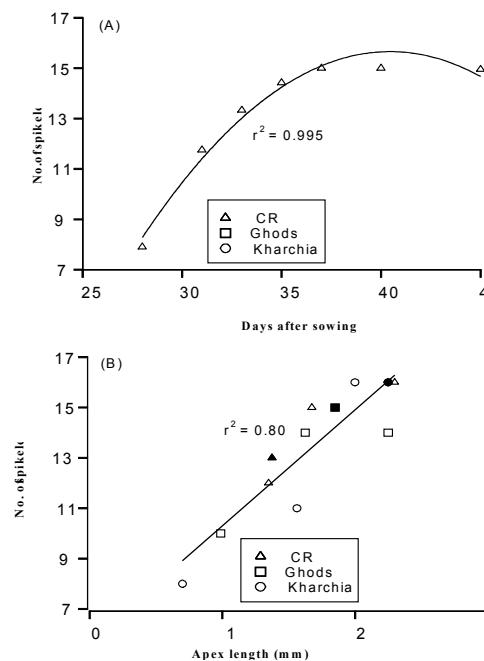
double ridges stage (20-25 DAS). During the period of spikelet initiation (25-40 DAS), there were no differences between salinity and control plants in apex length, but at 35 DAS (10 days after starting of spikelet initiation), stressed plants had longer apex than that of control (Figure 1b).

A positive and significant correlation was observed between number of spikelets per apex and the duration of spikelet initiation, and the relationship shifted to linearity at terminal spikelet (Figure 2a). Number of spikelets had also a significant correlation with apex lengths until terminal spikelet formation (Figure 2b), but this correlation ended after this stage because spikelet initiation had ended, while apex elongation continued.

Salinity had a negative effect on the number of spikelet primordia per apex in differ-



**Figure 1:** Number of spikelet per apex, length of apex (mm) in the period between double ridges and terminal spikelet. Each point is the average of two measurements and three cultivars. Three lines are the average of four levels of salinity ( $\Delta$ ), control ( $\square$ ), and high salinity level ( $\circ$ ). Vertical lines are standard error of means at 95% probability.

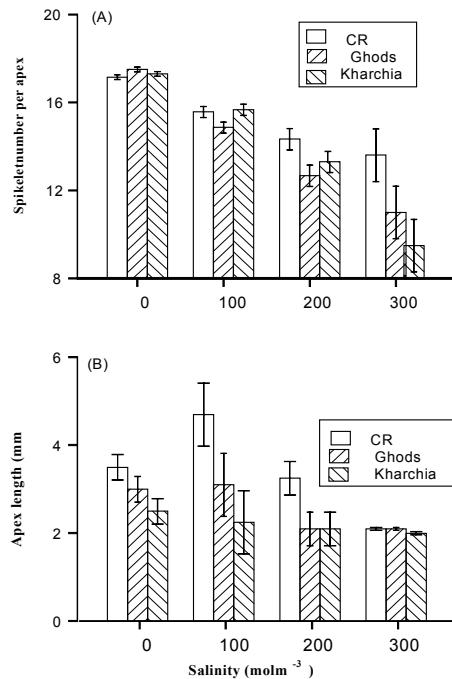


**Figure 2:** Relationship between number of spikelet primordia and time (a), number of spikelet primordia and apex length (b). Each point is the average of four levels of salinity and three cultivars (a) and seven replicate (b). Open symbols are salinity treatments and solid symbols are control (b).

ent cultivars. CR showed the highest spikelet number amongst cultivars at 200 and 300 mol m<sup>-3</sup> salinity levels (Figure 3a). In spite of salinity tolerance of cultivar Kharchia-65, its spikelet number at 300 mol m<sup>-3</sup> was significantly lower as compared with CR, whereas at zero salinity level all three cultivars did not differ significantly for spikelet number.

The apex length at the time of terminal spikelet formation followed the trend for spikelet number (Figure 3b). The apex length of cultivar CR was significantly longer than those of other cultivars only at 100 and 200 mol m<sup>-3</sup>. Spikelet initiation rate did not show any reduction due to salinity up to 200 mol m<sup>-3</sup> in all cultivars. The rate of spikelet initiation for all cultivars was reduced with the exception of CR (data not shown).

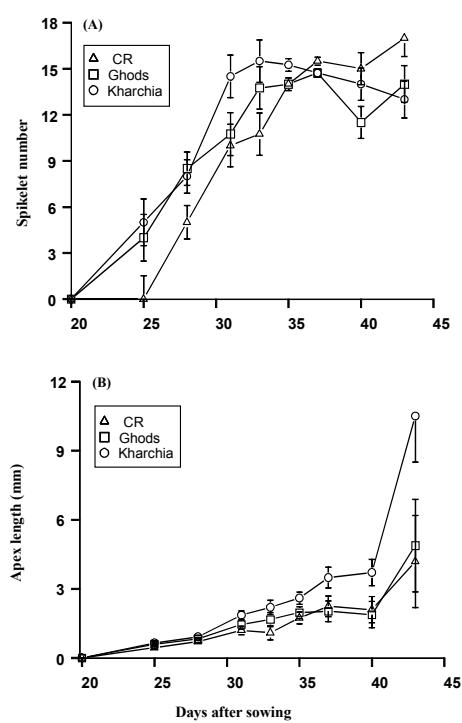
Total spikelet number per apex of CR was significantly higher than those of the other two cultivars but its apex developmental stage was well behind (Figure 4a). Apex



**Figure 3.** Number of spikelet per apex (a) and apex length (mm) (b) in various wheat cultivars at different levels of salinity. Each point is the average of two replicates. Vertical lines are standard error of means of cultivars at 95% probability.

length had a sharp increment after terminal spikelet stage, as during five days (40- 45 DAS), the apex length of cultivar Kharchia-65 and Ghods grew from 1.88 and 3.71 to 4.88 and 10.5 mm, respectively (Figure 4 b). Apex length was always higher in Kharchia-65 than in the other cultivars during the last four sampling dates, but based on developmental stage, CR had the longest apex at the time of terminal spikelet initiation. For instance, although the apex length of Kharchia-65 and CR at 40 days after sowing were 3.71 and 2.2 mm, respectively (Figure 4 ), at terminal spikelet stage they had the apex lengths of 2.2 and 3.4 mm, respectively.

Salinity shortened the duration of double ridge to terminal spikelet in all three cultivars. For instance, this period under non-saline conditions in cultivar Ghods was 13.5 days, whereas, at 200 mol m<sup>-3</sup> salinity it was 8.5 days (Table 1). Kharchia-65 reached its double ridge and terminal spikelet stages



**Figure 4.** Relationship between number of spikelet and time after sowing (a) and apex length with time after sowing (b) in different wheat cultivars in the presence of salinity. Each point is the average of two replicates and four levels of salinity. Vertical lines are standard error of means of cultivars at 95% probability.

earlier than others. It achieved its terminal spikelet stage, 33 days after sowing in overall levels of salinity, whereas Ghods and CR reached this stage after 37 and 45 days, respectively (table 1). It should be pointed out that the average duration of apex development of Ghods was longer than other cultivars (Table 1).

## DISCUSSION

Maximum number of spikelet primordia that can be produced on the wheat apex is determined by genotype, and this potential is realized by the interaction between genotype and environment (Rahman *et al.* 1978). In this experiment, final spikelet number in the main stem of wheat cultivars was affected with increasing salinity. Different cultivars

showed different responses to each level of salinity. The two main parameters which determined spikelet number per apex are duration and rate of spikelet initiation. Both of these factors were affected by salinity. Duration of apex development from double ridge to terminal spikelet was reduced in the presence of salinity (table 1). Double ridge stage commenced earlier in stressed plants than control by at least one day per 100 mol m<sup>-3</sup> mixture of NaCl and CaCl<sub>2</sub>. Spikelet initiation in salt-treated plants also completed earlier than that in control plants (table 1). These results are in agreement with Maas and Grieve (1990) and Grieve *et al.*, (1993) who reported that salinity caused a reduction in duration of the spikelet primordium initiation phase. The rate of spikelet initiation showed an increase in mild salt concentration as compared with control, but at the highest level of salinity this rate was reduced. This result does not agree with Oosterhuis and Cartwright (1983) who reported that there was no correlation between the length of the spike initial and the number of spikelet primordia formed in water-stressed wheat plants. The slight increase in spikelet initiation rate at 100 mol m<sup>-3</sup> salinity was not due to increasing the number of spikelet, but rather due to reduction in duration of spikelet initiation.

Although at high salinity, both the duration and rate of spikelet initiation were reduced, reduction in the rate of spikelet initiation was relatively more than the duration. These results are inconsistent with those of Grieve *et al.* (1993) who found that rate of spikelet initiation did not change with salinity. Numerous investigators have addressed the interaction between genotype and environment that influence final spikelet number. An inverse relationship between the rate and duration of spikelet initiation has been observed in response to some environmental stresses such as photoperiod (Allison and Daynard, 1976; Rahman and Wilson, 1977b; Rahman *et al.* 1978), irradiance (Allison and Daynard, 1976), temperature (Friend, 1965; Rahman and Wilson, 1977a), nutrient supply (Whingwiri and Kemp, 1980; Frank and



**Table 1:** Effect of salinity on duration of spikelet initiation, starting of double ridges (DR), and terminal spikelet (TS) of wheat cultivars. The numbers in the table are in days for duration, and days after sowing for the rest. Salinity is based on mol m<sup>-3</sup> in the nutrient solution, and 0 indicates the control. SE is the standard error of means in 95% probability.

Salinity	Cultivar	Experiment 1			Experiment 2			
		DR	TS	Duration	DR	TS	Duration	Average
0	CR	29	39	10	31	43	12	11.0
0	Ghods	22	34	12	25	40	15	13.5
0	Kharchia	26	35	9	25	40	15	12.0
100	CR	29	38	9	27	37	10	9.5
100	Ghods	25	31	6	25	34	9	7.5
100	Kharchia	25	34	9	25	33	8	8.5
200	CR	27	34	7	28	35	7	7.0
200	Ghods	23	31	8	24	33	9	8.5
200	Kharchia	25	31	6	24	31	7	6.5
300	CR	27	35	8	31	37	6	7.0
300	Ghods	21	34	11	23	33	10	10.5
300	Kharchia	25	31	6	23	31	8	7.0
SE		1.8	1.2	1.2	1.0	2.77	1.66	1.3

Bauer, 1984), and water stress (Frank *et al.*, 1987). However, the relationship between rate and duration does not appear to be strictly reverse and these factors may operate independently in the determination of spikelet number (Rahman and Wilson, 1977a).

The three wheat cultivars used in the present study showed different developmental time-tables from sowing to terminal spikelet differentiation (table 1). Shorter duration of apex growth phase, and the lower spikelet number production in Kharchia-65 (standard salt-tolerant) compared to CR and Ghods might be due to weakness of plants at the time of double ridges in this cultivar. Although in all three cultivars, the duration of the spikelet initiation phase and the number of spikelets formed were reduced under saline conditions compared to control. Statistical analysis revealed that in cultivar CR reduction in final spikelet number was lower than those in other cultivars. This result indicates that CR is resistant to salt stress as compared to Ghods and Kharchia-65 during this period of development. A better performance of the cultivar CR could be due to

a longer period between salinization and start of spikelet initiation which was two to six days later than those in the other cultivars, allowing treated plants in cultivar CR to adapt to the saline conditions and establish more vigorous plants at the double ridge stage (table 1).

However, many researchers (Kirby, 1974; Baker and Gallagher, 1983; Delecolle *et al.* 1989) have reported that spikelet primordia are initiated two to four times faster than leaf primordia. In all treatments, apex length was very small at the end of spikelet initiation phase (*i.e.* less than 3.5 mm). Apex length was shorter in stressed plants by the time of terminal spikelet formation (end of spikelet initiation) as compared with control. But as shown in figure 1b, salt-treated plants initiated their terminal spikelet before control plants, and because of rapid elongation of apex after this stage, recovery for apex length in stressed plants had taken place even earlier than terminal spikelet differentiation in control plants. It can be said that in stressed plants terminal spikelet initiation ended earlier and consequently more photosynthetic source was available for apex

elongation (figure 1b). Elongation of the apex was rapid after terminal spikelet initiation in all cultivars and salt treatments. Therefore, the start of rapid elongation of spike could be a good indicator for completion of apex development.

In summary, spikelet number and apex length both declined in the presence of salinity as compared with control. Both shorter duration and reduced rate of spikelet initiation were responsible for the reduction in spikelet number in salt-treated plants. Amongst cultivars, CR showed a better performance in spikelet initiation stage, which in turn produced relatively more spikelets under saline conditions than the other two cultivars. More information into the causes of the behavior of the wheat cultivars grown under salinity stress should be sought.

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## نمودار اولیه سه رقم گندم در شرایط سوری

### چکیده

به منظور درک چگونگی نمو سنبله اولیه ارقام مختلف گندم در سطوح مختلف شوری، دو آزمایش متواالی در محیط رشد کنترل شده در اتاقک رشد انجام شد. سه رقم گندم بهاره به نامهای کراس روشن ( مقاوم به شوری)، قدس (حساس به شوری) و خارچیای - ۶۰ ( مقاوم به شوری استاندارد ) در محیط رشد شن پرورش یافتند. چهار سطح شوری صفر، ۱۰۰، ۲۰۰، ۳۰۰ مول در مترمکعب با استفاده از املاح کلرور سدیم و کلرور کلسیم به نسبت مولی پنج به یک همراه با محلول غذایی هوگلنند اصلاح شده اعمال گردید. جوانه سنبله ساقه اصلی به منظور اندازه گیری طول سنبله اولیه و تعداد آغازی سنبله در جوانه سنبله جدا گردید. در این آزمایشها تعداد نهایی سنبله در ارقام گندم با افزایش سطح شوری کاهش یافت. ارقام مختلف نیز پاسخهای متفاوتی به سطوح شوری نشان دادند. دو پارامتر عمده تعیین کننده تعداد آغازی سنبله در جوانه سنبله دوام و سرعت تمایز سنبله ها بودند. تداوم نمو جوانه سنبله از مرحله برآمدگی دوگانه تا تولید سنبله انتهایی در حضور شوری کاهش یافت. دوره کوتاهتر و سرعت کمتر تمایز سنبله ها عوامل اصلی کاهش تعداد سنبله در سنبله گیاهان تحت تنش شوری بودند. در بین ارقام گندم، کراس روشن در مرحله تمایز سنبله ها مقاومت بیشتری نشان داده و تعداد سنبله بیشتری در شرایط سوری نسبت به ارقام دیگر تولید نمود.