

## **Impacts of waterlogging on shoot apex development and recovery effects of nitrogen on grain yield of wheat**

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### **ABSTRACT**

*The waterlogging as an abiotic stress can damage to crops such as wheat during winter season. Any suitable crop management during the growth and development of this plant can reduce yield loss due to waterlogging stress. This study was carried out to investigate the effects of different periods 10, 20 and 30 days waterlogging and without waterlogging stress conditions, at the time of the double ridge emergence and adding different levels of reparative nitrogen fertilizer, respectively, 0, 50, 100 and 150 kg ha<sup>-1</sup> at end of stress duration, to reduce waterlogging damage on winter wheat (*Triticum aestivum* var. *Marvdasht*). An experiment factorial based on Randomized Complete Block Design, with three replications was conducted in 2009-2010 cropping season at the research field of college of agriculture, Razi University, Kermanshah, Iran. The results showed that the studied traits i.e., spikelet number, apical meristem length, rate of spikelet initiation and also rate of spikelet elongation (based on day and growth degree days), during double ridge appearance to terminal spikelet formation were significantly influenced by waterlogging stress, where the most negative effect was recorded for 30 days waterlogging treatment. The grain yield, the number of spike m<sup>-2</sup>, the number of grains per spike, the grain weight per spike and the biological yield were also affected by waterlogging stress. According to the results, for each day of waterlogging, a reduction equal to 1.5% was obtained for grain yield. The effect of reparative fertilizer (except for 1000-kernel weight) was significant for other traits. In conclusion, by application the reparative fertilizer the grain yield increased and waterlogging damage was reduced.*

**Keywords:** nitrogen, shoot apex, waterlogging stress, wheat

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### **INTRODUCTION**

Waterlogging has affected about 10 percent of the global land areas and 1 million hectares of the sown areas in the Iran [12]. It is a stress that limiting growth and development of many plant species in natural and agricultural ecosystems [9]. Heavy soil texture (as clay), high rainfall, heavy irrigation, poorly drained soil and low slope of the land are all causes of waterlogging stress [8, 20, 5, 1]. The lake of oxygen is the main cause of stress for plants under waterlogging conditions [6, 21]. In crops, damage of waterlogging dependent on temperature, the growth stage of plant, duration of waterlogging, cultivar and water surface elevation [4, 10, 19]. The distractive effects of waterlogging at prior growth stages of wheat are more than later growth stages of it [15, 25]. The results of research

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show that, the wheat is susceptible to flooding and this sensitivity depends on growth stage of wheat, duration of waterlogging stress and cultivar [20, 24]. However, there are many ways for prevent or reduce of destructive effects of waterlogging on wheat. The effect of waterlogging is less under nutrient supply conditions [16]. Application of nitrogen fertilizer after waterlogging has been shown to reduce the detrimental effects of this stress [27]. Also, understanding the critical stage of growth and physiology of the crop (i.e., wheat) can be effective in better management of it. Duration double ridge stage to terminal spikelet is one of the important stages in wheat. In fact, appearance of double ridge is indication of transition of the wheat from vegetative phase to reproductive phase [3]. Because differentiation of spikelets and florets occurs at this stage and terminal spikelet marks the end of initiation of spikelet primordia and thus potential grain set [26]. Whatever duration and rate of double ridge to terminal spikelet in shoot apex of the wheat be more, the final yield of it also, will be more. Various reports indicate that various environmental factors including salinity [17], water stress [7], nutrient supply [29], photoperiod [22], and temperature [23], have been effective on the apex development of wheat.

The main objectives of this study were determine effects of waterlogging stress during the period of double ridge to terminal spikelet stage on the shoot apex in winter wheat (Marvdasht cultivar) and understanding this point that whether application of reparative nitrogen fertilizer after removing stress can be effective on the recovery of the yield and yield related traits or not?

## MATERIALS AND METHODS

### Experimental data

In this study, a factorial experiment was conducted in 2009-10 cropping season at the Research Farm of Campus of Agriculture and Natural Resources of Razi University, Kermanshah (coordinates: 34° 21' N and 47° 9' E; and with elevation of 1319 AMSL), Iran. The factors consisted of (i) different periods 10, 20 and 30 days waterlogging and non-waterlogging stress (control) conditions at time of the double ridge emergence and (ii) adding different levels of reparative nitrogen fertilizer 0, 50, 100 and 150 kg ha<sup>-1</sup> (urea source), in the end of the waterlogging stress duration to reduce waterlogging damage. The experiment was carried out based on RCBD with three replications on winter wheat (cv. Marvdasht). This cultivar is one of the winter wheat that has optimum yield and area cropping in the Kermanshah province. The planting density was 400 seed m<sup>-2</sup>. To prevent entering water from each plot to another the distance between plots 2 meter was considered. More information on soil is given in (Table 1). The crop received 498.3 mm rainfall during its growth and the average annual temperature maximum and minimum were 22.6 °C and 5.9 °C, respectively.

### The measured traits

#### a) Apical meristem (shoot apex)

After emergence of double ridge stage (Table 2), three main shoots were measured from each plot. The developed spikelets were investigated by a quantitative scale [28]. The main shoot apex was dissected to determine the apex lengths and the number of spikelet primordia present on the apex. The number of spikelet buds and apex lengths were measured using a binocular microscope, at a magnification of X40 (Fig 1). The double unit of leaf primordium and spikelet bud counted as one [18]. The rate of apex elongation and the rate of spikelet initiation were expressed per day and growth degree days (GDD). The spikelet initiation rate ( $\beta$ ), and the apex elongation rate ( $\alpha$ ), were calculated by dividing the number of spikelets (S) and length of the apex (L) (mm), on duration between double ridge (D<sub>1</sub>), and terminal spikelet (D<sub>2</sub>), on the basis of day and growth degree days [17].

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

$$\alpha = L / (D_2 - D_1) \rightarrow (\text{mm/day}), (\text{mm/GDD})$$

**Table 1. Characteristics of the soil which the experiment was conducted**

Soil depth (cm)	Soil texture	Soil particles (%)			Organic carbon (%)	Organic matter (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	N (%)	Lime (%)	pH
		Clay	Silt	Sand							
0-30	Silty-clay	52.0	46.0	2.0	1.1	1.8	8.6	410.0	0.1	33.0	7.9
30-60	Silty-clay	54.0	45.0	0.0	1.1	1.8	7.8	390.0	0.1	32.0	7.8

#### b) The yield and the yield components

At harvest stage, one square meter of each plot was considered to measure of the traits of biological yield, grain yield and number of spike. Number of grains per spike was also calculated from 20 spikes per plot.

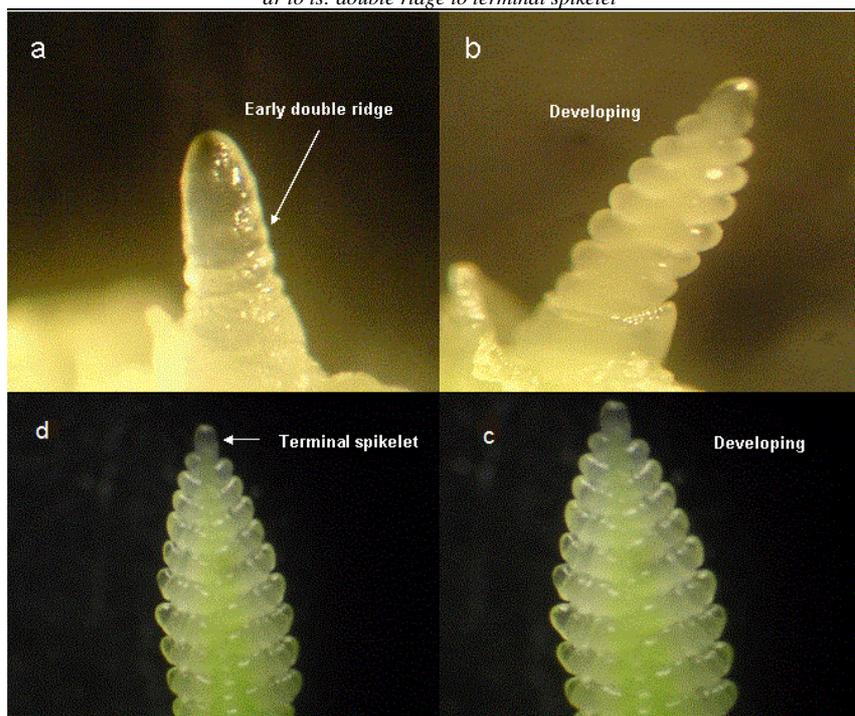
#### c) Grain protein

The percentage of grain protein was determined by Kjeldahl method [2].

The all analyses i.e., normal test, analysis of variance and mean comparisons were performed by using the statistical softwares of SPSS 16.0 and SAS 9.1.

Developmental stages	Date (time)	Total GDD (from dr to ts)
Sowing	November 16, 2009	-
3-leaf	Jan 7, 2010- Feb 24, 2010	-
Double ridge	Feb 24, 2010	-
Terminal spikelet		-
Plants without waterlogging	March 15, 2010	152.0
Plants under 10 days waterlogging	March 16, 2010	169.0
Plants under 20 days waterlogging	March 18, 2010	193.5
Plants under 30 days waterlogging	March 20, 2010	208.0
Anthesis	April 26, 2010	-
Harvesting	June 16, 2010	-

*dr to ts: double ridge to terminal spikelet*



**Figure 1.** Double ridge initiation (a), developing of apex (b) and (c) and terminal spikelet appearance (d). Photos provided by author and belonging to this experiment

## RESULTS

### 1. Apical meristem traits

The effect of waterlogging on the spikelet initiation number (in main shoot), at during double ridge to terminal spikelet was significant (**Table 3**). The highest average of the spikelet initiation number (22 spikelets) was observed for control condition (no waterlogging stress) and the lowest one (20 spikelets) was found at 30 days waterlogging stress (**Table 4**). Although the range of spikelet formation was not considerable, there was significant difference between the treatments. The trait of the spikelet initiation number had a negative relationship with the days of waterlogging (**Fig 2b**).

S. O. V	Df	Spikelet initiation number	Apex length	Spikelet initiation rate (day)	Spikelet initiation rate (GDD)	Apex elongation rate (day)	Apex elongation rate (GDD)
Waterlogging	3	8.3*	0.5**	94.4**	61.3**	316.7**	312.3**
C. V%	-	2.3	2.6	2.4	4.3	2.3	2.8

*ns, non-significant; \* and \*\* significant at the 5% and 1% levels of probability, respectively*

**Table 4. Mean comparisons of wheat apical meristem traits during double ridge to terminal spikelet stage**

Waterlogging periods (day)	Spikelet initiation number	Apex length (mm)	Spikelet initiation rate day <sup>-1</sup>	Spikelet initiation rate GDD <sup>-1</sup>	Apex elongation rate day <sup>-1</sup>	Apex elongation rate GDD <sup>-1</sup>
non- waterlogging	22.3 <sup>a</sup>	3.6 <sup>a</sup>	1.1 <sup>a</sup>	0.14 <sup>a</sup>	0.18 <sup>a</sup>	0.024 <sup>a</sup>
10 d waterlogged	21.7 <sup>ab</sup>	3.3 <sup>b</sup>	1.0 <sup>b</sup>	0.12 <sup>b</sup>	0.15 <sup>b</sup>	0.019 <sup>b</sup>
20 d waterlogged	21.3 <sup>b</sup>	2.9 <sup>c</sup>	0.9 <sup>c</sup>	0.10 <sup>c</sup>	0.12 <sup>c</sup>	0.014 <sup>c</sup>
30 d waterlogged	20.3 <sup>c</sup>	2.7 <sup>d</sup>	0.8 <sup>d</sup>	0.09 <sup>d</sup>	0.10 <sup>d</sup>	0.012 <sup>d</sup>
LSD	0.99	0.16	0.04	0.01	0.007	0.001

Means at least one common letter in each column, based on Least Significant Difference (LSD) test at 5% level are not significant

The length of apex was significantly influenced by waterlogging stress (**Table 3**). The difference in length equal to 0.3 mm made a significant difference between the days of the waterlogging and control conditions (**Table 4**). The highest (3.6 mm) and lowest (2.7 mm) rates of the apex length during double ridge to terminal spikelet, corresponding to control conditions and 30 days waterlogging, respectively (**Table 4**). There was a negative relationship between the apex length and the days of waterlogging (Fig. 2a) while the relationship between apex length and the number of spikelet initiation was positive (**Fig 2c**).

The effect of waterlogging stress on the spikelet initiation rate (based on day and GDD), was significant (**Table 3**). The apex elongation rate (based on day and GDD), significantly influenced by waterlogging treatments (**Table 3**). The highest and the lowest rate of apex elongation rate belong to control condition and 30 days waterlogging stress. A significant difference was found between the periods of waterlogging (**Table 4**).

## 2. Yield and Yield components

The main effects of the waterlogging and the reparative nitrogen fertilizer were found to be significant for grain yield, but the interaction effects were not significant (**Table 5**). The maximum reduction of the grain yield between the periods of waterlogging was belonged to the 30 days waterlogging stress (**Table 6**), and no application of the reparative nitrogen fertilizer (**Table 7**). For each day of waterlogging, there was 1.5% reduction in the grain yield (**Table 8**).

The effect of waterlogging on the number of spikes m<sup>-2</sup> was found to be significant (**Table 5**), and it considerably influenced by the level of 30 days waterlogging stress (**Table 6**). For each day of waterlogging stress, the reduction of number of spikes m<sup>-2</sup> was 0.7 % (**Table 8**). The effect of the reparative nitrogen fertilizer on the number of spike was significant, while the interaction of the waterlogging and the reparative nitrogen fertilizer wasn't significant (**Table 5**).

The main effects of the waterlogging and the reparative nitrogen fertilizer for number of grains per spike were also significant (**Table 5**). The ratio of the yield loss for each day waterlogging stress was 0.9 % (**Table 8**), and the ratio of recovery of this reduction was 0.2 % for one kg ha<sup>-1</sup> application of the reparative nitrogen fertilizer (**Table 9**).

The effects of the waterlogging, the reparative nitrogen and the interaction between of them for grain weight per spike were also found to be significant (**Table 5**). The plants under waterlogging stress (particularly over 10 days waterlogging) were wrinkled and had small grains than those under control condition. In this study, there was a reduction equal to 1.3 % was observed for grain weight per spike for each day waterlogging stress (**Table 8**).

The effects of the waterlogging, the reparative nitrogen fertilizer and their interaction on the 1000-grains weight were not significant (**Table 5**).

The main effects of the waterlogging and the reparative nitrogen for biological yield were significant (**Table 5**). The lowest and the highest amounts of biological yield were obtained at 30 days waterlogging and control conditions, respectively (**Table 6**), while for reparative nitrogen fertilizer the lowest (8962.3) and the highest (13178.9 kg ha<sup>-1</sup>) amounts were obtained for no application and the level of 150kg ha<sup>-1</sup> (**Table 7**). The ratio of the biological yield for each day waterlogging was 1.2% (**Table 8**), and the ratio of recovery of this reduction was 0.31% for each kg ha<sup>-1</sup> application of the reparative nitrogen fertilizer (**Table 9**).

The main effects of the waterlogging and the reparative nitrogen fertilizer on the grain protein percent were significant, but the interaction between of them was not significant (**Table 5**).

**Table 5. Analysis of Variance for yield and yield components of wheat**

S. O. V	df	Grain yield	Number of spikes m <sup>-2</sup>	Number of grains per spike	Grain weight per spike	1000-grains weight	Biological yield	Grain protein
W	3	71.3**	16.6**	12.4**	17.0**	2.8 <sup>ns</sup>	56.3**	57.3**
RN	3	8.7**	4.1*	9.9**	12.6**	2.7 <sup>ns</sup>	35.8**	5.5**
Interaction W × RN	9	0.8 <sup>ns</sup>	0.1 <sup>ns</sup>	1.8 <sup>ns</sup>	2.3*	1.4 <sup>ns</sup>	0.9 <sup>ns</sup>	0.1 <sup>ns</sup>
C.V. (%)	-	8.1	8.1	12.4	17.9	17.9	9.6	5.1

ns, non-significant; \* and \*\* significant at the 5% and 1% levels of probability, respectively

W: Waterlogging RN: Reparative Nitrogen

**Table 6. Mean comparisons for traits at different period of waterlogging stress**

Waterlogging periods (day)	Grain yield (kg ha <sup>-1</sup> )	Number of spikes m <sup>-2</sup>	Number of grains per spike	Grain weight per spike (g)	1000-grains weight (g)	Biological yield (kg ha <sup>-1</sup> )	Grain protein (%)
non-waterlogging	7518.4 <sup>a</sup>	489.7 <sup>a</sup>	45.0 <sup>a</sup>	1.8 <sup>a</sup>	40.3 <sup>a</sup>	13473.2 <sup>a</sup>	10.9 <sup>a</sup>
10	6815.5 <sup>b</sup>	439.0 <sup>b</sup>	39.2 <sup>b</sup>	1.4 <sup>b</sup>	35.3 <sup>ab</sup>	12312.4 <sup>b</sup>	10.2 <sup>b</sup>
20	5587.0 <sup>c</sup>	427.7 <sup>b</sup>	38.1 <sup>b</sup>	1.3 <sup>b</sup>	35.2 <sup>ab</sup>	9599.0 <sup>c</sup>	9.4 <sup>c</sup>
30	4138.6 <sup>d</sup>	388.0 <sup>c</sup>	33.1 <sup>c</sup>	1.1 <sup>c</sup>	32.9 <sup>b</sup>	8541.7 <sup>d</sup>	8.5 <sup>d</sup>
LSD	506.4	29.7	4.0	0.2	5.4	884.1	0.4

Means at least one common letter in each column, based on Least Significant Difference (LSD) test at 5% level are not significant

**Table 7. Mean comparisons traits at different levels of reparative nitrogen**

Reparative nitrogen ha <sup>-1</sup> (kg)	Grain yield (kg ha <sup>-1</sup> )	Number of spikes m <sup>-2</sup>	Number of grains per spike	Grain weight per spike (g)	1000-grains weight (g)	Biological yield (kg ha <sup>-1</sup> )	Grain protein (%)
0	5261.8 <sup>b</sup>	411.5 <sup>c</sup>	34.3 <sup>c</sup>	1.1 <sup>c</sup>	31.4 <sup>b</sup>	8962.3 <sup>d</sup>	9.4 <sup>c</sup>
50	6077.2 <sup>a</sup>	429.9 <sup>bc</sup>	37.0 <sup>bc</sup>	1.3 <sup>b</sup>	36.9 <sup>a</sup>	10132.1 <sup>c</sup>	9.6 <sup>bc</sup>
100	6353.9 <sup>a</sup>	442.1 <sup>ab</sup>	39.3 <sup>b</sup>	1.4 <sup>b</sup>	37.7 <sup>a</sup>	11652.9 <sup>b</sup>	9.9 <sup>ab</sup>
150	6368.5 <sup>a</sup>	461.0 <sup>a</sup>	44.7 <sup>a</sup>	1.7 <sup>a</sup>	37.8 <sup>a</sup>	13178.9 <sup>a</sup>	10.1 <sup>a</sup>
LSD	506.4	29.7	4.0	0.2	5.4	884.1	0.4

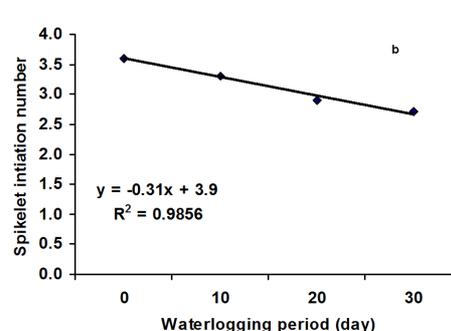
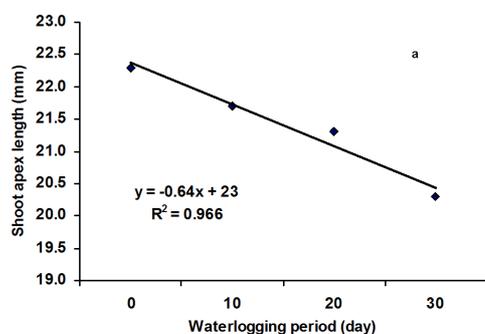
Means at least one common letter in each column, based on Least Significant Different (LSD) test at 5% level are not significant

**Table 8. Reduce of yield and yield components of wheat at different periods of waterlogging relative to without waterlogging conditions (control) (%)**

Waterlogging periods (day)	Grain yield	Number of spikes m <sup>-2</sup>	Number of grains per spike	Grain weight per spike	1000-grains weight	Biological yield	Grain protein
Without waterlogging	100	100	100	100	100	100	100
10	90.7	89.6	87.1	77.8	87.6	91.4	93.6
20	74.3	87.3	84.7	72.2	87.3	71.2	86.2
30	55.0	79.2	73.5	61.1	81.6	63.4	78.0

**Table 9. Increase of yield and yield components of wheat at different levels of reparative nitrogen in wheat relative to non-use of it (%)**

Reparative nitrogen (kg ha <sup>-1</sup> )	Grain yield	Number of spikes m <sup>-2</sup>	Number of grains per spike	Grain weight per spike	1000-grains weight	Biological yield	Grain protein
0	0	0	0	0	0	0	0
50	15.5	4.5	7.9	18.2	17.5	13.0	2.1
100	20.7	7.4	14.6	36.4	20.1	30.0	5.3
150	21.0	12.0	30.3	54.5	20.3	47.0	7.4



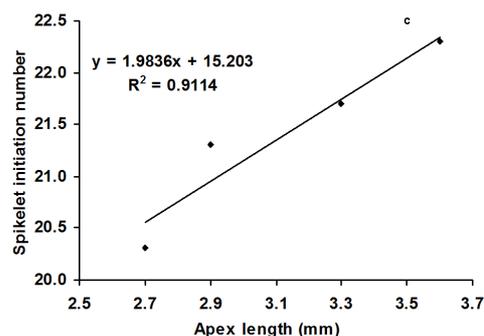


Figure 2. Relationships between apex length and periods of waterlogging (a), spikelet initiation number and periods of waterlogging (b), and apex length and spikelet initiation number (c)

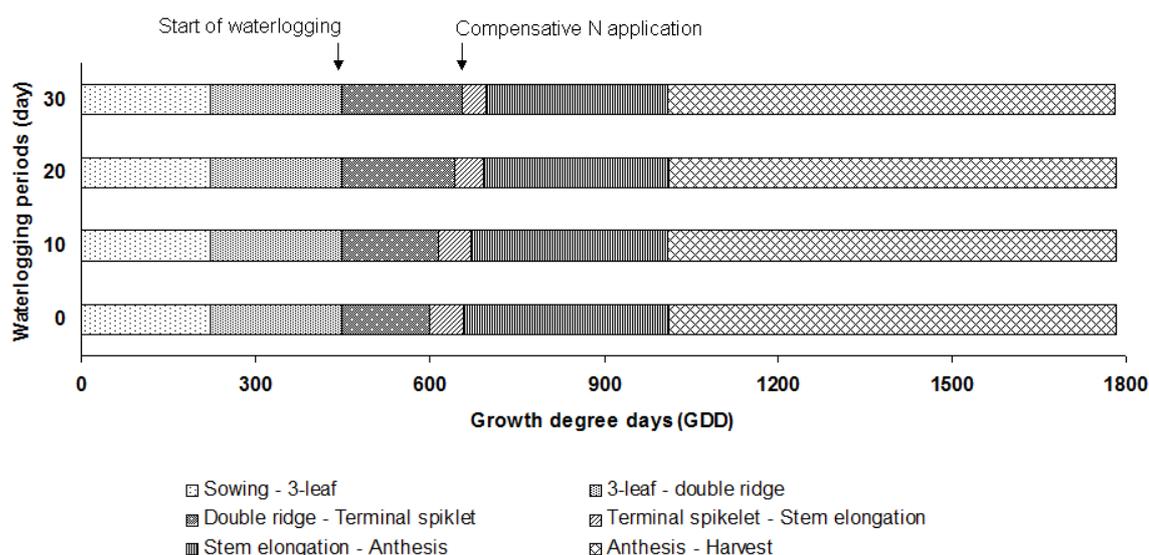


Figure 3. During the developmental stages of the wheat according to GDD values

## DISCUSSION

According to the results, the effect of waterlogging stress for apical meristem, which studied during double ridge to terminal spikelet stage, was found to be significant. For some traits such as the rate of spikelet initiation (based on day and GDD), the apex length and the rate of elongation (based on day and GDD), even short-term period of 10 days waterlogging could make a significant difference than the control (without waterlogging conditions), but period of 30 days waterlogging had the greatest negative effect on them. **Fig. 3** which shows duration of the developmental stages of wheat based on growth degree days (GDD), indicates that, duration of developmental stages of wheat from sowing to prior of waterlogging stress for all of plots has been the same, but with applying waterlogging, time of the transition from double ridge to terminal spikelet appearance between the plants is different. As shown in **Fig. 3**, the duration of this phase is less for non-stress condition, namely, they have received less than amount of GDD for transition from double ridge to terminal spikelet stage. With increasing duration of waterlogging, the plants have received more GDD. So that, the period of 30 days waterlogging have been received the maximum amount of GDD. This result is true for number of days for transition from double ridge to terminal spikelet (**Table 2**). After the end of the waterlogging stress, and with adding the reparative nitrogen fertilizer (i.e., Urea), to the plants, this (received different of GDD), at period of terminal spikelet to stem elongation was found between plants, but the difference was not much as previous period. This is considerable and important point that, the duration of the terminal spikelet to the stem elongation was a bit more in the plants that were under non-waterlogging conditions than those who were under waterlogging. The longer period in which all plants were under non-stress but received different levels of the reparative nitrogen fertilizer was identical. Namely, the reparative nitrogen fertilizer didn't have effect on the amounts of GDD, that received by plants.

Also, there was a certain synchronicity at the times of the anthesis and the harvesting between plants that were under different periods of the waterlogging and those who received different levels of the reparative nitrogen fertilizer in a special period of the waterlogging.

The results of the yield and the yield components show that, there was a significant difference between all of the periods of the waterlogging. Although, 30 days waterlogging had more negative effects on the grain yield. However, the period of 10 days waterlogging could also make a significant difference with non-waterlogging conditions. So, the short periods of waterlogging had negative effect on the grain yield of the wheat, but the most negative effect related to 30 days waterlogging. This is also true for traits of the biological yield and the grain protein. Ghobadi and Ghobadi [13], in the assessment of the effects of different waterlogging duration at different growth stages of some wheat cultivars, found that, grain yield and biological yield of the wheat were decreased 44.5 and 39.8%, respectively. They showed, even 10 days waterlogging had distractive effects on the grain yield of the wheat. Reduction of the grain yield of wheat under waterlogging already reported by others [8, 30, 14, 11].

The negative effect of this stress was reduced with application of the reparative nitrogen fertilizer after removing of the waterlogging stress, but this reduction wasn't associated with the use of more fertilizer, because only application of 50 kg ha<sup>-1</sup> of the reparative nitrogen fertilizer compared with non-use of it, had more compensatory effect (15.5 %), than the application of 100 and 150 kg ha<sup>-1</sup> of reparative nitrogen (20.7 and 21.0 percent respectively). But this point was different for trait of the biological yield. For instance, with more application of the reparative nitrogen fertilizer, the ratio of increasing of the biological yield was greater.

### CONCLUSION

Although, longer transition from double ridge to terminal spikelet and rate of spikelet initiation caused improve the yield of the wheat (due to improving of yield components), but in this experiment, the wheat crop that had longer transition from double ridge to terminal spikelet, had lower grain yield. This reduction of the yield related to reduce or stop the growth of the wheat. Furthermore, with application of the reparative nitrogen fertilizer, the grain yield of the wheat improved and in this experiment the application of 50 kg ha<sup>-1</sup>, reparative nitrogen fertilizer for recover of the growth and grain yield of the wheat, after removing waterlogging stress is recommended.

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