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EFFECT OF AUXIN AND NITROGEN ON SEED YIELD AND GERMINATION TRAITS OF PRODUCED WHEAT SEEDS

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Abstract: *Field and laboratory experiments were conducted to assess the response of wheat to external auxin application and nitrogen. Studied factors in the field experiment were different concentrations of auxin (0, 50, and 100 ppm) and nitrogen fertilizer application (0, 75, and 150 kg/ha). At the laboratory experiment, germination traits of produced seeds from maternal plants under the field experiment treatments were assessed. Results showed that no auxin application with nitrogen application had higher stem and leaf yield than auxin application of 50 ppm with nitrogen application of 150 kg/ha. Under high nitrogen application, the negative effect of external auxin on seed yield occurred at a lower concentration. Under nitrogen application of 75 kg/ha, auxin application of 100 ppm reduced harvest index and seed weight. Nitrogen application reduced seed germination percentage but auxin broke the negative effect of nitrogen on seed germination.*

Key words: *maternal plant environment, nitrogen, phytohormone, seed vigour.*

1. Introduction

Due to the increase in population and the growing need for crop production, the use of chemical fertilizers has become inevitable. Many researchers have studied the effect of nitrogen on wheat growth and yield [11, 25, 26]. In one study, nitrogen split application increased grain yield and 1000-grain weight of wheat [14]. In another study, nitrogen split application increased wheat grain yield and protein content [7].

Plant hormones are substances that in small amounts have tremendous effects on plant growth. Auxin has been considered by researchers as a growth hormone more than other plant hormones [8, 9]. Application of naphthalene acetic acid at a rate of 90 ml/ha at the emergence stage of rice panicle had the highest number of panicles, spikelet number per panicle, seed yield, and 1000-seed weight [2]. Application of indole butyric acid and gibberellic acid at the flowering stage of maize (*Zea mays*)

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increased the yield and yield components of the plant compared to the control [4]. Treatment of 100 ppm indole acetic acid, 100 ppm gibberellic acid, and 15% coconut juice increased plant height, stem diameter, and leaf development in *Hibiscus sabdariffa* L. [19]. The use of 2,4-Dichlorophenoxyacetic acid (2,4-D) + 2-methyl-4-chlorophenoxyacetic acid (MCPA) at different stages of wheat growth, including the beginning and end of tillering, the beginning of stalk emergence, and before heading showed that there was a difference in grain yield, 1000-grain weight, and biological yield between the use of 2,4-D + MCPA and its non-use [23]. Application of indole acetic acid and naphthalene acetic acid at the rate of 100 and 200 mg/l at the early stages of carrot (*Daucus carota*) growth increased the secondary umbel number. Seed yield and seed quality also increased due to the increase in the secondary umbel number. Improvement of seed quality can be due to increased activity of antioxidant enzymes and phenol content of seed [20]. In a study to investigate the effect of 2,4-D and Gibberellic acid (GA3) on the fruit retention of *Averrhoa carambola* L., it was observed that using 10 ppm 2,4-D, 60 ppm GA3 + 5 ppm 2,4-D or 60 ppm GA3 increased fruit weight per cluster [10].

Wheat seeds were soaked in 2,4-D and MCPA solution and then germinated. Caulicle and radicle growth of wheat increased by low concentrations (0.001 to 1 ppm) and decreased by high concentrations (100 and 1000 ppm) [22]. To combat salinity, wheat seedlings were pretreated with 10 µM 2,4-D herbicide for 48 hours. The results showed that the treated seedlings had higher growth, biomass, and water content due to the decrease in sodium accumulation and increased absorption of calcium, magnesium, and potassium and the 2,4-D herbicide has the potential to be used to control salinity [17]. Coconut water extract improved the seedling shoot and root length in maize, rice, and several legumes compared to the control. Chemical analysis of this extract showed that it contained indole acetic acid, gibberellic acid, abscisic acid, cytokinin, zeatin, and kinetin [15]. With the increase of nitrogen in the environment of *Centaurea cyanus* mother plant, the germination percentage of the resulting seeds decreased [16]. Use of 100 kg N/ha compared to non-use reduced the average emergence time of rapeseed [21].

Nitrogen as the most important fertilizer used in agriculture can be involved in increasing the strength of the source (leaf area) and sink strength (spike) of wheat. The interaction of auxin and nitrogen is likely to increase source and sink strength. Research on the impact of the mother plant environment on seed production is limited. Therefore, this study was designed to determine the germination characteristics of seeds from the mother plant and find the best hormonal and fertilizer treatment to increase wheat grain yield.

2. Materials and Methods

2.1. Field Experiment

Before planting in November 2013, the land located in Chamchamal plain of Kermanshah was ploughed with a mouldboard plough. Simultaneously with planting, urea fertilizer and triple superphosphate fertilizer were mixed with soil. Urea and triple superphosphate fertilizers were applied at a rate of 250

kg/ha. Of course, half of the urea fertilizer was applied at the beginning of the stem emergence on April 24. Wheat (*Triticum aestivum* L.) seeds of Pishtaz cultivar with 98% germination were prepared from Biston Rural Service Center. Seeds were sown by hand with a density of 280 kg/ha. In the spring, after the weeds emerged, herbicides were used to control the field weeds. The most important weeds in the field were *Phragmites sp., Galium verum, Avena sp., Sinapis arvensis, Myagrom sp.,* and *Polygonum aviculare.* In spring, the field was irrigated three times using surface irrigation and well water. Special pesticides were used to control the pest of wheat. Wheat rust was also a common disease of wheat that showed symptoms on the leaves.

The studied treatments included different concentrations of auxin (0, 50, and 100 ppm) and the application of nitrogen fertilizer (0, 75, and 150 kg N/ha). The auxin hormone used was 2,4-D + MCPA, which was sprayed on the aerial parts of the plant using a back sprayer at the spike emergence stage of wheat (May 15, 2014). Auxin concentrations were calculated based on 400 litres of water used per hectare of tractor sprayers. The nitrogen fertilizer used was also from a urea source which was top-dressed on the soil surface at the same time with auxin at the spike emergence stage and before irrigation. The experiment was performed as a split-plot design with three replications. Auxin was considered as the main plot and nitrogen as the subplot. Experimental plots were considered 1.5 * 1.5 m^2. The distance between the plots was one meter and the distance between the replications was two meters. At the time of harvest (June 20, 2014), three plants per plot were selected and the traits of stem and leaf weight, number of seeds per spike, spike length, spike weight, seed yield, single seed weight, biological yield, and harvest index were calculated.

2.2. Laboratory Experiment

In the laboratory study, the germination characteristics of seeds from the mother plant under the auxin and nitrogen were evaluated. For this purpose, 15 wheat seeds were placed in sterile Petri dishes after disinfection with sodium hypochlorite. The experiment was performed in a completely randomized design with three replications. Six ml of distilled water was poured into each Petri dish. The resulting Petri dishes were covered by a plastic bag to prevent moisture evaporation and placed in a germinator at 20°C for one week. After one week, germination characteristics including germination percentage, radicle and caulicle length, and seed vigour were calculated. Two millimetres of caulicle growth was considered as the germination criterion. Seed vigour was obtained by multiplying seedling length (total radicle and caulicle length) by germination percentage [5].

2.3. Data Analysis

Data were analyzed by variance and means were compared using Duncan's multiple range test. Correlation between traits was also obtained. SAS, MINITAB, and SPSS statistical software were used for this purpose. Correlation coefficients between traits were calculated by Pearson method. Data were normalized before the analysis of variance.

3. Results and Discussion

3.1. Field Experiment

3.1.1. Stem and Leaf Yield and Spike Weight

The results showed that non-application of auxin with nitrogen application had higher stem and leaf yield and spike weight than treatment of 50 ppm auxin with 150 kg N/ha (Figures 1a and 1b). It seems that the content of internal auxin in this study was sufficient for plant growth and the application of external and additional auxin had no effect on increasing the growth of vegetative and reproductive parts of the plant (stem, leaves, and spike) and sometimes even reduced the growth of these parts. 2,4-D foliar application on cowpea did not affect the weight of the pod produced, but the foliar application of coconut juice increased the weight of the pod compared to no foliar application [18].

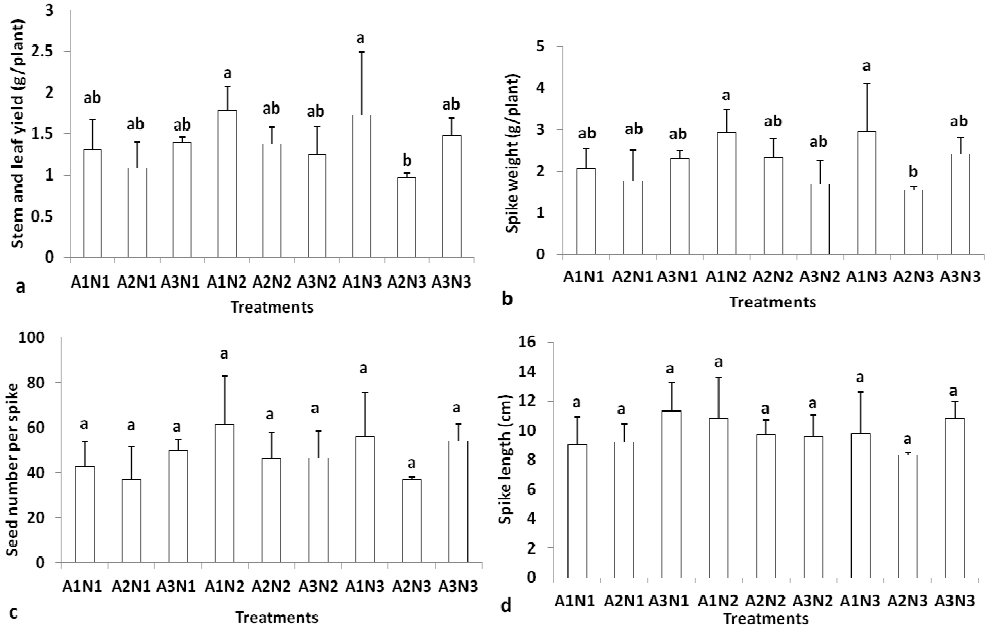

Fig. 1. *Effect of auxin and nitrogen rates on wheat traits: a. stem and leaf yield; b. spike weight; c. seed number per spike; d. spike lenght. Values are mean (three replicates) + standard deviation* (A1, A2, and A3 represent auxin application of 0, 50, and 100 ppm, respectively. N1, N2, and N3 represent nitrogen application of 0, 75, and 150 kg/ha, respectively. Means with the same letter in each trait are not significantly different according to Duncan's Test ($P < 0.05$))

3.1.2. SEED Number per Spike and Spike Length

Nitrogen and auxin treatment did not affect the number of seeds per spike and spike length in wheat (Figures 1c and 1d). Traits such as the number of seeds per spike and spike length at the spike emergence stage are almost determined, and the application of treatments such as nitrogen and auxin cannot change these traits much. The number of seeds per

spike is determined at the pollination stage [6]. Due to the self-pollination of wheat and the time interval between the application and effect of the treatments studied in this experiment, it can be said that the number of seeds was determined at the time of treatment application and only seed weight might be changed. Ghodrat et al. [4] reported a change in ear length and number of grains per ear of maize (*Zea mays*) under gibberellic acid and indole butyric acid treatments. The longer growth period of the reproductive stage of maize than wheat can be a possible reason for the difference in the results.

3.1.3. Seed Yield

When 75 kg N/ha was applied, non-application of auxin produced higher seed yield than the application of 100 ppm auxin and when 150 kg N/ha was applied, non-application of auxin produced higher seed yield than the application of 50 ppm auxin (Figure 2a). These results indicate that when high nitrogen is used, the negative effect of external auxin on seed yield occurs at lower concentrations. Regarding that the experimental treatments were applied at the stage of spike emergence, the content of internal auxin in the plant may be sufficient for seed formation and growth, and the excess auxin may act as a growth inhibitor in some way by increasing concentration [9]. Spraying 50 ppm 2,4-D had heavier and taller cob and ear than spraying 25 ppm auxin and spraying other herbicides (atrazine and glyphosate). This treatment also had a higher green forage yield and baby corn yield (14%) than the control. The treatment time was 25 and 45 days after sowing [24]. The difference between the results of the present study and this study may be related to the difference between the stages of treatment. Seed yield had a positive and significant correlation with all studied traits (Table 1).

Table 1

Correlation coefficients between wheat traits in auxin and nitrogen rates

	Spike length (SL)	Spike weight (SW)	Stem and leaf yield (SLY)	Seed number per spike (SNS)	Seed yield (SY)	Seed weight (SW)	Biological yield (BY)	Harvest index (HI)
SL	1	0.668**	0.741**	0.832**	0.549**	-0.144	0.703**	-0.056
SW		1	0.944**	0.902**	0.980**	0.539**	0.993**	0.565**
SLY			1	0.897**	0.906**	0.395*	0.977**	0.354
SNS				1	0.844**	0.174	0.912**	0.355
SY					1	0.653**	0.966**	0.683**
SW						1	0.493**	0.848**
BY							1	0.495**
HI								1

*, ** - Significance at the level of 0.05 and 0.01, respectively.

3.1.4. Seed Weight

The highest seed weight was related to the application of 150 kg N/ha with no nitrogen application (A1N3) and the lowest seed weight was related to the application of 75 kg N/ha with the application of 100 ppm auxin per hectare. Non-use of nitrogen along with consumption of 100 ppm auxin also had less seed weight than A1N3. These results indicate a reduction in wheat seed weight when high auxin is consumed (Figure 2b).

Foliar application of naphthalene acetic acid at a concentration of 90 ml ha^{-1} at the panicle formation stage produced the highest grain weight of rice (*Oriza sativa*) among different concentrations (0 to 120 ml ha^{-1}) and a concentration of 120 ml at the grain formation stage was not different from the control (no foliar application) [2]. Treatment of 400 mg l^{-1} naphthalene acetic acid increased the diameter and weight of *Citrus unshiu* fruit and increased its marketability [1].

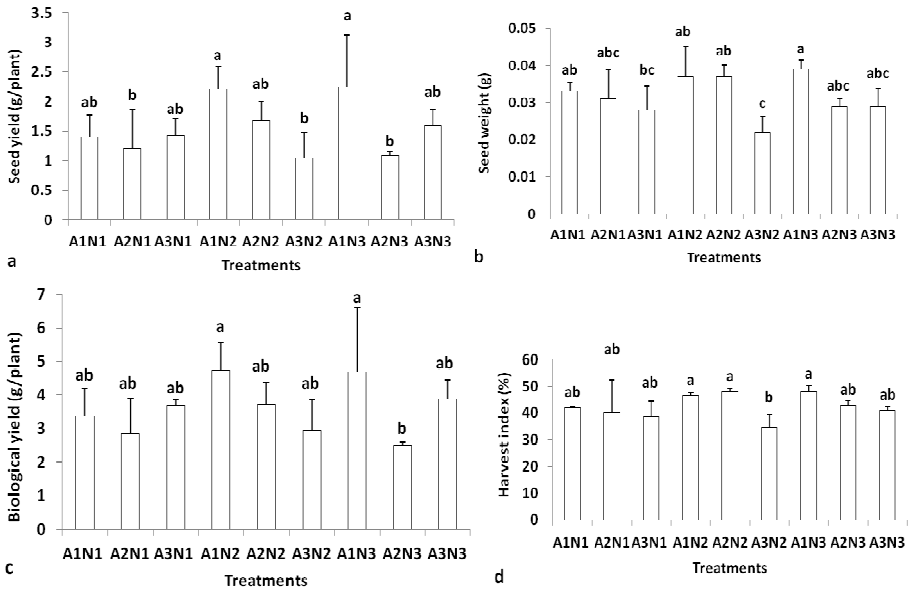

Fig. 2. *Effect of auxin and nitrogen rates on wheat traits: a. seed yield; b. seed weight; c. biological yield; d. Harvest index. Values are mean (three replicates) + standard deviation* (A1, A2, and A3 represent auxin application of 0, 50, and 100 ppm, respectively. N1, N2, and N3 represent nitrogen application of 0, 75, and 150 kg/ha, respectively. Means with the same letter in each trait are not significantly different according to Duncan's Test ($P < 0.05$))

3.1.5. Biological Yield

Under 150 kg N/ha, application of 50 ppm auxin had a lower biological yield than non-auxin application (Figure 2c). Non-application of auxin with the application of 75 kg N/ha also had a higher biological yield than the application of 150 kg N/ha with the application of 50 ppm auxin. These results indicate that the

adverse effect of external auxin on biological yield becomes apparent in conditions where a large amount of nitrogen is consumed. High nitrogen may reduce the need for auxin or growth hormone by increasing plant growth. Among the various hormonal treatments (abscisic acid, kinetin, and indole acetic acid), not only indole acetic acid but also other treatments increased the plant dry matter compared to the control [3]. A positive correlation of biological yield with all studied traits (Table 1) is due to that the studied traits are a component of biological yield.

3.1.6. Harvest Index

In conditions where 75 kg of nitrogen per hectare was used, consumption of 100 ppm auxin reduced the harvest index (Figure 2d). Considering that the seed weight of wheat also decreased in the 100 ppm auxin treatment when 75 kg of nitrogen per hectare was consumed, it can be said that high auxin has reduced the harvest index by reducing the seed weight. The positive correlation of seed weight with harvest index confirms this (Table 1).

3.2. Laboratory Experiment

In conditions where 150 kg N/ha was applied, the non-auxin application had the lowest germination percentage (Figure 3a). In other words, consumption of auxin in these conditions produced seeds with a high germination percentage. These data also show that under non-auxin application, consumption of high levels of nitrogen in the mother plant reduced the germination percentage of seeds to less than half. These results show that nitrogen consumption reduces seed germination percentage and is consistent with the results of other researchers [12] but auxin eliminates the negative effect of nitrogen on seed germination. Excessive nitrogen in the seed may induce dormancy and seed auxin with hormonal changes will disrupt this dormancy and stimulate seed germination. In one study, plant treatment with 2,4-D increased the levels of the hormones abscisic acid, salicylic acid, and 2,4-D but decreased ethylene production [13]. The correlation of seed germination percentage with other seed germination characteristics was positive (Table 2).

Table 2

Correlation coefficients between wheat seed traits in auxin and nitrogen rates

	Germination percent (GP)	Radicle length (RL)	Caulicle length (SL)	Seed vigour (SV)
GP	1	0.514**	0.198	0.853**
RL		1	0.615**	0.802**
SL			1	0.569**
SV				1

*, ** - Significance at the level of 0.01.

The length of caulicle and radicle of seeds of mother plant under different concentrations of auxin and nitrogen were not different (Figures 3b and 3c). Under non-auxin application, the application of high nitrogen reduced wheat seed visor compared to the non-nitrogen application (Figure 3d). This result shows the negative

effect of high seed nitrogen on seedling vigour during germination. The reduction of seed vigour under the influence of nitrogen also showed itself through the reduction of seed germination in the correlation relationship (Table 2). However, the possible presence of auxin in the seeds eliminates this negative effect. The use of coconut water extract on seedlings increased the length of shoot and root in some crops. This extract contained indole acetic acid [15]. Of course, in our study, the seeds of the mother plant were studied, which part of the difference in the results goes back to this issue.

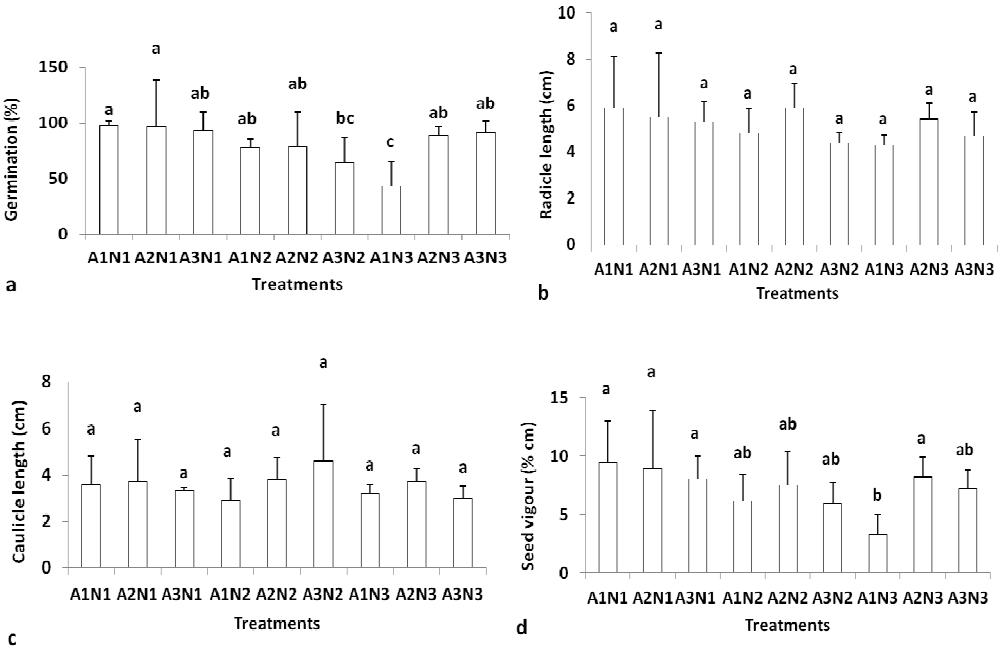

Fig. 3. *Effect of auxin and nitrogen rates on wheat seed traits: a. seed germination; b. radicle length; c. Caulicle length; d. seed vigour. Values are mean (three replicates) + standard deviation* (A1, A2, and A3 represent auxin application of 0, 50, and 100 ppm, respectively. N1, N2, and N3 represent nitrogen application of 0, 75, and 150 kg/ha, respectively. Means with the same letter in each trait are not significantly different according to Duncan's Test ($P < 0.05$))

4. Conclusions

Under conditions where a lot of nitrogen was used, the negative effect of external auxin on seed yield occurred at a lower concentration. At a rate of 75 kg N/ha, the application of 100 ppm auxin reduced the harvest index and seed weight. Non-auxin application had the lowest germination percentage when 150 kg N/ha was applied. In other words, application of auxin in these conditions produced seeds with a high germination percentage. The study of the interaction of auxin and nitrogen at other stages of wheat growth, especially before the onset of reproductive growth, should be considered in future studies.

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