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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Alteration of Wheat Source-Sink Relation by Nitrogen and Spikelet Removal

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Abstract

The source and sink relationships determine the amount and distribution of biomass in plants. Field and laboratory experiments were conducted to study the effect of nitrogen rate and spikelet removal on seed yield and germination traits of wheat. The field experiment was conducted employing sink manipulation (no spikelet removal and ½ spikelet removal) and source manipulation (nitrogen rate of 0, 75, and 150 kg ha^{-1}). This study was performed as a factorial experiment in a randomized complete block design with three replications. Seeds obtained from the field experiment were subjected to determine the effect of the sink and source manipulation on seed germination traits in the laboratory experiment. Results showed that most traits under study were not affected by source and sink manipulation. Seed yield and seed weight were not affected by spikelet removal and varying nitrogen applications. Although some of the wheat spikelets have been removed, those plants have been able to maintain the number of seeds per spike and the weight of a single seed. The use of nitrogen at the spike emergence stage did not affect the seed yield of the Pishtaz cultivar. Nitrogen needed for the seeds could be compensated by the re-mobilization of nitrogen from various plant organs such as the stem. Nitrogen application of 150 kg ha^{-1} with the removal of ½ spikelets improved seed germination (%) and vigor comparing control (no spikelet removal with no nitrogen application). Considering that seed yield and seed weight did not change under the influence of source and sink manipulation, it can be concluded that Pishtaz wheat is more sink-limited than source-limited.

Keywords: Biomass, Germination, Harvest index**,** Plant nutrition**,** Seed vigor, *Triticum aestivum* L.

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1. Introduction

In crops, the organ in which food is made is called the source and the place where food is stored is called the sink. If the sink is small, the plant yield will not be high. Even if the sink is large but the source is limited, the yield will be low (Smith et al., 2018). More dry matter accumulation in some new wheat cultivars is associated with a higher allocation of photosynthate to the reproductive parts of the plant, which increases by more nitrogen uptake from the soil (Zhang et al., 2020). Nitrogen fertilization significantly increased grain yield in wheat. Nitrogen applied in spring and split-application of nitrogen led to higher seed yield than in autumn application (Ghimire et al., 2021). Elimination of old fruits decreased the respiration rate and increased the sugar content of new fruits (Zhou et al., 2000). In maize, the removal of 50% of the cob due to sink limitations had minimal remobilization (Falihzade et al., 2013). Uhart and Andrade (1991) reported that maize grown in the temperate zone had both sink and source constraints. While Seebauer et al. (2010) reported that in maize, grain compounds have source limitations. Mohammadi et al. (2014) reported that there is more sink limitation in new wheat and work needs to be done to increase the sink capacity.

In two tomato cultivars, photosynthesis was not affected by the source-sink change, but the net assimilation rate of tomatoes increased with an increasing source-sink ratio (Matsuda et al., 2011). In a study of the relationship between the source and the sink of three wheat varieties, it was observed that the single-grain weight decreased with the removal of the flag leaf and penultimate leaf and increased with the removal of the spikelet. This shows that wheat grain yield is more source-limited than sink-limited and more attention should be paid to increasing the source (Xiao-li et al., 2022). Manipulation of wheat source and sink showed that removal of half spikelets caused an increase, but defoliation caused a decrease in single grain growth. These changes show that the studied wheat cultivar has both sink and source limitations (Lv et al., 2020). Manipulating the relationship between source and sink with methods such as nitrogen fertilizer and trimming in wheat genotypes did not affect grain weight, but it affected grain quality (Arata et al., 2023). Ma et al. (1996) observed that the removal of wheat spikelets increased the grain weight of the responsive cultivars, but there was no difference in final grain weight between 50 and 75% removal of spikelets. In the study of the relationship between the source and the sink, it was observed that the removal of half of the spikelets caused a decrease in the number of grains in the spike, the weight of a single spike, and the weight of the whole grain of wheat (Wang et al., 2021). In the conditions of drought stress, the removal of wheat spikelets increased grain yield and 1000-grain weight by 35 and 21%, respectively (Abdoli et al., 2013). In maize, leaf removal resulted in significant remobilization of stem reserves, which did not completely alleviate seed weight loss. Seed weight was more sensitive to decreasing source-sink ratio than green leaf area duration (Abeledo et al., 2020). In canola, partial removal of pods and complete leaf removal at full flowering reduced pods per plant and yield. Supplementary irrigation increased yield without affecting the average grain weight. Canola yield is limited by the size of the sink at the flowering stage and by the source at the grain-filling stage (Zhang and Flottmann, 2018). Nitrogen is one of the most consumed nutrients that can be involved in the power of the source. Nitrogen consumption increased yields in crops such as wheat (Akdag and Zengin, 2020) and soybean (*Glycine max* (L.) Merrill) (Basal and Szabo, 2020).

The maternal plant environment can affect the germination characteristics of produced seeds (Kathleen, 2009; Sales et al., 2013; Sánchez et al., 2022). In *Sinapis arvensis*, it was observed that nitrogen enrichment of the maternal plant environment reduced the germination percentage of produced seeds, which is probably due to the induction of dormancy in the seeds (Luzuriaga et al., 2006). The addition of nitrogen to the maternal plant environment in *Potentilla tanacetifolia* significantly affected seed production, seed weight, and seed germination (Li et al., 2011). Environmental conditions during sunflower seed growth on the mother plant regulated seed dormancy rate. Seed dormancy at harvest time was a function of growth cycle length and seed drying rate on the mother plant (Lachabrouilli et al., 2021). Adding nitrogen to the medium of the mother plant increased the germination percentage and decreased the germination time of the Perennial Grass *Leymus chinensis* (Trin.) Tzvel seeds (Zhao et al., 2021).

There are limited studies on the effect of source and sink constraints on the germination characteristics of crop seeds. Therefore, this research was designed to determine the seed yield and germination characteristics of wheat seeds under varying source and sink relationships.

2. Materials and Methods

2.1. Field experiment

The field experiment was conducted on Chamchamal Plain located 40 km from Kermanshah (latitude 34 degrees north, longitude 47 degrees east, and altitude 1300 meters above sea level). The mean annual precipitation in the region is 442 mm (IMO, 2012). Chamchamal Plain has a high groundwater level and the groundwater level in April reaches about 1.5 meters from the soil surface (Razzaghmanesh et al., 2004).

2.1.1. Agronomical practices

The land used last year was under maize cultivation. In the fall of 2012, the land was plowed by a moldboard plow. Then phosphorus fertilizer at the rate of 266 kg ha^{-1} was used. Wheat (*Triticum aestivum*) seeds of the Pishtaz cultivar with 98% germination at the rate of 333 kg ha^{-1} were sown manually on November 10th. This cultivar was introduced by the Cereal Research Department, Seed and Plant Improvement Institute, Karaj, for cultivation in the temperate climate of Iran. Pishtaz wheat is relatively late and somewhat sensitive to plant loading and high yield. In spring, at the beginning of stem elongation, 300 kg ha^{-1} of urea fertilizer was applied as a top dressing. The most important weeds were wild oats (*Avena sp*), wild mustard (*Sinapis arvensis*), lady's bedstraw (*Galium verum*), and reed (*Phragmites sp*.). 2, 4-D + MCPA herbicide was used to control broad-leaved weeds. Wheat sunn pest was one of the pests observed in the field which was controlled with a suitable pesticide. During the growing season, the plants were irrigated three times (spike emergence stage, grain milk stage, and grain dough stage) by the surface method.

2.1.2. Experimental design and sampling

This study was performed as a factorial experiment in a randomized complete block design with three replications. The size of the plots was one square meter and the distance between the plots was considered to be one meter and the distance between the repetitions was two meters. The time of the wheat spike's emergence was May 10, 2013. The time of applying the treatments was May 15th. Experimental treatments included sink manipulation (no spikelet removal and removal of half spikelets) and source manipulation (consumption of 0, 75, and 150 kg N ha^{-1}) at the spike emergence stage. The spikelets were removed with a cutter. Nitrogen was used as a top dressing. The amounts of nitrogen used at the spike emergence stage are in addition to the amounts used at the start of stem elongation.

At the time of harvest (June 27), three plants per plot were randomly selected. These plants represented that plot. Spike length, spike weight, stem and leaf weight, number of seeds per spike, single seed weight, seed yield, and biological yield were scored. The biological yield was obtained from the total weight of the leaf, stem, and seed. Plant samples were weighed on a digital scale. A ruler with millimeter precision was used to measure the spike length. To measure the weight of a single seed, firstly 100 seeds were selected and weighed, then divided by 100. The harvest index was computed by dividing seed yield by biological yield.

2.2. Laboratory experiment

This study was designed to investigate the effect of the maternal plant environment (source and sink manipulation) on the germination characteristics of seeds obtained from the field experiment. After separating the seeds from the mother plant, the seeds were kept at room temperature for one week. This study was conducted at Razi University as a factorial experiment in a completely randomized design with three replications in July 2013. Wheat seeds were sterilized with 1% sodium hypochlorite solution for 10 minutes and washed immediately with water. Twenty wheat seeds were placed on a filter paper in each disinfected Petri dish. 8 mm of sterile distilled water was poured into each Petri dish and the Petri dishes were kept at 30°C for one week. Three seedlings per Petri dish were used to measure caulicle length, radicle length, seedling weight, and seed vigor. To measure the seed germination percentage, seeds that had two millimeters of caulicle growth were counted as germinated. The germination percentage was obtained by dividing the number of germinated seeds by all the seeds inside each Petri dish. Seed vigor was calculated using Heidari et al. (2013) equations (Eq. 1, 2).

$$\text{Seed vigor based on length } (\%\ \text{cm}) = \text{Seed germination percentage} \times \text{Seedling length (cm)} \qquad (\text{Eq. }1)$$
$$\text{Seed vigor based on weight } (\%\ \text{g}) = \text{Seed germination percentage} \times \text{Seedling weight (g)} \qquad (\text{Eq. }2)$$

In the above equations, the seedling length is the sum of the radicle length and the caulicle length. The seedling weight is the sum of the radicle weight and the caulicle weight.

2.3. Statistical analysis

MINITAB statistical software was used to find outlier data and test normality. The data were first analyzed by SAS statistical software and PROC GLM procedure. Then Duncan's test was used at the 5% probability level to compare the mean of the data. SPSS statistical software was used to calculate correlation coefficients between traits using Pearson's method.

3. Results and Discussion

3.1. Field experiment

Variance analysis of data showed that nitrogen and removal of spikelets had no significant effect on stem and leaf weight, spike weight, seed yield, seed weight, biological yield, and harvest index in wheat (*Table 1*). The removal of half spikelets had a significant effect on the number of seeds per spike and the length of the spike in wheat (*Table 1*).

3.1. 1. Stem and leaf weight and spike length

A comparison of the mean data showed that there was no difference between the studied treatments in terms of stem and leaf weight (*Table 2*). Probably the weight of the stem and leaves reached its maximum weight at the stage of spike emergence, and from this stage onwards, the reproductive part should have gained weight. The length of the spike was reduced by removing half of the spikelets (*Table 2*). This is perfectly normal because half of the spike is removed at the time of flowering. Nitrogen consumption of 75 kg ha^{-1} improved the spike length compared to nitrogen consumption of 150 kg ha^{-1}. Excess nitrogen may stimulate the growth of vegetative parts of the plant, although in this study the difference in stem and leaf weight was not significant. She et al. (2023) reported that nitrogen consumption of more than 150 to 240 kg per hectare did not affect wheat grain yield and growth. Spike length had a significantly positive correlation with most of the studied traits (*Table 3*). Spike length had a significantly negative correlation with seed weight (*Table 3*). This indicates that the seed weight increases with a decreasing number of seeds per spike.

3.1.2. Number of seeds per spike and weight of spike

Removing half of the florets only when nitrogen was not used or a small amount of nitrogen (75 kg ha^{-1}) was used, reduced the number of seeds per spike compared to treatment of 75 kg N ha^{-1} with no spikelet removal (*Table 2*). Spike's weight was not affected by any of the treatments. It seems that if, at the early stages of spike emergence, stress occurs, the plant loses some of its seeds, and the Pishtaz cultivar can compensate. Source constraints at the time of wheat seed filling reduced the number of seeds per unit area (Uhart and Andrade, 1991). The number of seeds per spike and the weight of the spike had a significantly positive correlation with most of the studied traits (*Table 3*). The number of seeds per spike had a significantly negative correlation with seed weight (*Table 3*). This indicates that the seed weight increases with a decreasing number of seeds per spike.

3.1.3. Seed yield and seed weight

Seed yield and seed weight were not affected by experimental treatments (*Table 2*). It seems that although some of the wheat florets have been removed, this plant has been able to maintain the number of seeds per spike and the weight of a single seed, which, ultimately, seed yield was not affected by treatments. The use of more nitrogen at the spike emergence stage did not affect the seed yield of the Pishtaz cultivar. Perhaps the plant provided the nitrogen needed for the seeds by re-mobilization nitrogen from organs such as the stem or soil nitrogen was enough for the seeds to grow. It is not possible to say here which factor (source or sink) limits the yield of the Pishtaz cultivar because seed yield has not changed under the influence of these two factors. Uhart and Andrade (1991) reported that source limitation during wheat grain filling reduced grain weight. In the study of the relationship between the source and sink in rice, it was seen that increasing the ratio of spikelet to leaf led to more allocation of nitrogen and biomass to the spike instead of the leaf and caused an imbalance between the source and the source (Li et al., 2023). Seed yield had a significantly positive correlation with most traits except seed weight and stem and leaf weight (*Table 3*).

3.1.4. Biological yield and harvest index

Biological yield and harvest index were not affected by source and sink manipulation (*Table 2*). The formed biomass of wheat may have been relatively completed at the spike emergence stage and at this stage, there has been a re-mobilization of material from the vegetative parts to the reproductive parts. Respiration of this cultivar may have lost part of the current photosynthetic reserves of the leaves, or it can be said that the current photosynthesis of the plant was not to the extent that it caused a significant increase in plant dry matter. However, some carbohydrates are lost by re-remobilizing material from vegetative parts such as the stem, which act as temporary food storage. Removal of wheat spikelets did not increase sucrose flow and starch accumulation in the remaining grains (Jenner, 1980), which is consistent with the results of our study. The harvest index had a significantly positive correlation only with seed yield and was not correlated with other traits (*Table 3*). This indicates that the harvest index is more important than other traits in increasing plant yield, and plant breeding work to manipulate the source and sink of wheat can focus on this trait.

Table 1. Analysis of variance (mean square) of the effect of spikelet removal and nitrogen on wheat traits

Source of variation	df	Stem and leaf weight	Seed number per spike	Spike length	Spike weight	Seed yield	Seed weight	Biological yield	Harvest index
Block	2	0.052^{*}	57.4^{ns}	2.28^{ns}	0.085^{ns}	0.089^{ns}	0.00003^{ns}	0.211^{ns}	164.7^{ns}
Spikelet removal (S)	1	0.042^{ns}	317.5^{*}	30.42^{**}	0.411^{ns}	0.228^{ns}	0.00007^{ns}	0.186^{ns}	239.8^{ns}
Nitrogen (N)	2	0.012^{ns}	25.7^{ns}	2.40^{ns}	0.019^{ns}	0.004^{ns}	0.00005^{ns}	0.030^{ns}	9.7^{ns}
S×N	2	0.001^{ns}	59.2^{ns}	2.22^{ns}	0.077^{ns}	0.049^{ns}	0.00003^{ns}	0.090^{ns}	3.4^{ns}
Error	10	0.011	36.6	1.44	0.132	0.072	0.00004	0.205	64.0

**,*=significant at the probability level of 1 and 5%, respectively. ^{ns}=non-significant

Table 2. Mean comparisons of wheat seed yield and yield-attributed traits under spikelet removal and nitrogen fertilizer

Treatments	Stem and leaf weight (g/plant)	Seed number per spike	Spike length (cm)	Spike weight (g/plant)	Seed yield (g/plant)	Seed weight (g)	Biological yield (g/plant)	Harvest index (%)
S1N1 ^{a}	0.632 ^{a}	29.3 ^{ab}	8.0 ^{ab}	1.53 ^{a}	1.08 ^{a}	0.036 ^{a}	2.16 ^{a}	49.8 ^{a}
S1N2	0.691 ^{a}	32.9 ^{a}	8.9 ^{a}	1.65 ^{a}	1.14 ^{a}	0.034 ^{a}	2.35 ^{a}	47.9 ^{a}
S1N3	0.708 ^{a}	22.9 ^{ab}	6.6 ^{bc}	1.33 ^{a}	0.96 ^{a}	0.044 ^{a}	2.04 ^{a}	50.0 ^{a}
S2N1	0.719 ^{a}	18.3 ^{b}	4.8 ^{c}	1.14 ^{a}	0.76 ^{a}	0.041 ^{a}	1.86 ^{a}	40.8 ^{a}
S2N2	0.781 ^{a}	19.9 ^{b}	5.5 ^{c}	1.18 ^{a}	0.81 ^{a}	0.043 ^{a}	1.96 ^{a}	41.1 ^{a}
S2N3	0.823 ^{a}	21. 7 ^{ab}	5.4 ^{c}	1.29 ^{a}	0.94 ^{a}	0.043 ^{a}	2.11 ^{a}	44.0 ^{a}

[a] S1 and S2 represent no spikelet removal and ½ spikelet removal, respectively. N1, N2, and N3 represent nitrogen applications of 0, 75, and 150 kg ha $^{-1}$, respectively.
[b] Means with the same letter in each trait have no significant difference according to Duncan's Test ($P < 0.05$).

3.2. Laboratory experiment

Analysis of the variance of the data showed that nitrogen and removal of spikelets had no significant effect on germination percentage, caulicle length, radicle length, and seedling weight of wheat (*Table 4*). The interaction effect of nitrogen and the removal of spikelets had a significant effect on the seed vigor based on weight of wheat (*Table 4*). Nitrogen had a significant effect on the seed vigor based on length of wheat (*Table 4*).

3.2.1. Seed germination percentage

A comparison of mean data showed that removing half of the florets with 150 kg N ha^{-1} had the highest germination percentage (*Table 5*). The removal of several florets may have increased the share of the remaining florets in obtaining the nutrients needed for seed growth, although this became significant when high nitrogen was available to the plant. Seed germination percentage is significantly positively correlated only with seed vigor (*Table 6*). Seed germination percentage is one of the components of seed vigor, so the correlation between these two traits has become significant. In the study of the effect of fertilization of wheat mother plant with four amounts of nitrogen (0, 50, 100, and 150 kg ha^{-1}), it was observed that 150 and 100 kg N ha^{-1} had the highest seed germination percentage and seed vigor, respectively (Paneru et al., 2017).

Table 3. Correlation coefficients between wheat seed yield and yield-attributed traits under spikelet removal and nitrogen

	Stem and leaf weight	Seed number per spike	Spike length	Spike weight	Seed yield	Seed weight	Biological yield	Harvest index
Stem and leaf weight	1	-.648	-.688	-.604	-.529	.684	-.320	-.651
Seed number per spike	-.648	1	.990**	.992**	.952**	-.881*	.922**	.730
Spike length	-.688	.990**	1	.977**	.943**	-.831*	.890*	.785
Spike weight	-.604	.992**	.977**	1	.981**	-.842*	.948**	.770
Seed yield	-.529	.952**	.943**	.981**	1	-.734	.953**	.834*
Seed weight	.684	-.881*	-.831*	-.842*	-.734	1	-.731	-.413
Biological yield	-.320	.922**	.890*	.948**	.953**	-.731	1	.652
Harvest index	-.651	.730	.785	.770	.834*	-.413	.652	1

$^{*, **}$. Significance at the level of 0.05 and 0.01

3.2.2. Caulicle and radicle length and seedling weight

A comparison of mean data showed that source and sink manipulation did not affect caulicle and radicle length and seedling weight (*Table 5*). Although the removal of some florets was expected to increase the chances of other florets absorbing nutrients and nitrogen consumption could increase caulicle and radicle length and seedling weight, these traits were not affected. There may have been enough nitrogen in the soil, or the plant may have had enough nitrogen storage in the vegetative organs during the spike stage, or nitrogen may not be absorbed from the soil at this stage. Caulicle length had a significantly positive correlation with radicle length and seedling weight (*Table 6*). The addition of nitrogen to the environment of the mother plant in *Potentilla tanacetifolia* significantly reduced the seedling weight produced by adding nitrogen to the seedling environment (Li et al., 2011).

Table 4. Analysis of variance (mean square) of the effect of spikelet removal and nitrogen on seed germination traits in wheat

Source of variation	df	Germination	Caulicle length	Radicle length	Seedling weight	Seed vigor (based on weight)	Seed vigor (based on length)
Spikelet removal (S)	1	830.0^{ns}	0.022^{ns}	0.005^{ns}	0.000003^{ns}	0.000019^{ns}	28.4^{ns}
Nitrogen (N)	2	885.6^{ns}	10.34^{ns}	1.770^{ns}	0.000008^{ns}	0.000026^{ns}	75.9*
S×N	2	1085.9^{ns}	3.75^{ns}	2.150^{ns}	0.000003^{ns}	0.000034*	22.1^{ns}
Error	12	277.7	3.218	2.685	0.000005	0.000008	9.5

*=significant at the probability level of 5%. ^{ns}=non-significant

3.2.3. Seed vigor

Under conditions where high nitrogen (150 kg ha^{-1}) was used and half of the florets were removed, seed vigor increased compared to the treatment in that the source and sink were not manipulated (*Table 5*). An increase in seed vigor based on length was also observed when the highest amount of nitrogen was used and spikelets were not removed. Seed vigor is a good indicator of seed germination, especially in adverse conditions such as dryland soils. Therefore, it can be said that a cultivar such as Pishtaz in terms of seed vigor has both source and sink limitations. The recommendation that can be made based on the results of this section is that it is necessary for the mother plants used to produce seeds to be supplied with nitrogen, even at the stage of spike emergence, to have seeds with high seed vigor. These results also confirm nitrogen restriction as the most important nutritional factor in plants. Therefore, it is necessary to use nitrogen as a split application even at the beginning of the reproductive stage. Heidari et al. (2013) reported that high nitrogen consumption with complete defoliation reduced seed vigor compared to control. The difference in results is probably due to the difference between defoliation and spikelet removal because, under defoliation conditions, the source is limited, but by removing part of the spikelets, sufficient photosynthetic material is provided for other spikelets.

Table 5. Mean comparisons of wheat seed germination traits under spikelet removal and nitrogen fertilizer

[a] Treatments	Germination (%)	Caulicle length (cm)	Radicle length (cm)	Seedling weight (g)	Seed vigor (%g)	Seed vigor (% cm)
S1N1 [a]	35.0 [b]	6. 6 [a]	9.2 [a]	0.013 [a]	0.0046 [b]	5.65 [b]
S1N2	65.0 [ab]	10.1 [a]	10.0 [a]	0.017 [a]	0.0111 [ab]	8.77 [ab]
S1N3	66. 7 [ab]	10.3 [a]	11.2 [a]	0.016 [a]	0.0106 [ab]	14.32 [a]
S2N1	68.3 [ab]	8.2 [a]	9.9 [a]	0.016 [a]	0.0106 [ab]	12.18 [ab]
S2N2	30.0 [b]	9.7 [a]	10.9 [a]	0.017 [a]	0.0048 [b]	5.23 [b]
S2N3	83.3 [a]	8.8 [a]	9.9 [a]	0.016 [a]	0.0130 [a]	15.62 [a]

[a] S1 and S2 represent no spikelet removal and ½ spikelet removal, respectively. N1, N2, and N3 represent nitrogen applications of 0, 75, and 150 kg ha $^{-1}$, respectively.
[b] Means with the same letter in each trait have no significant difference according to Duncan's Test ($P < 0.05$).

Table 6. Correlation coefficients between wheat seed germination traits under spikelet removal and nitrogen

	Germination percent	Caulicle length	Radicle length	Seedling weight	Vigor (based on weight)	Vigor (based on length)
Germination percent	1	.323	-.009	.312	.988**	.921**
Caulicle length	.323	1	.820*	.900*	.446	.320
Radicle length	-.009	.820*	1	.643	.075	.201
Seedling weight	.312	.900*	.643	1	.431	.226
Seed vigor (based on weight)	.988**	.446	.075	.431	1	.887*
Seed vigor (based on length)	.921**	.320	.201	.226	.887*	1

*, **: Significance at the level of 0.05 and 0.01

4. Conclusions

Manipulation of source and sink did not affect seed yield and seed weight, it can be concluded that the Pishtaz wheat is more sink-limited than source-limited. It is recommended that breeders focus on increasing the sink strength of Pishtaz wheat. It is also recommended to evaluate the effect of manipulation of source and sink by nitrogen and removal of spikelets on other wheat cultivars.

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