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Seed and Seedling Performance of Bread Wheat (*Triticum aestivum* L.) as Influenced by Rate and In-Season Nitrogen Application

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Authors' contributions

The research experiment was designed by joint effort of the two authors. Field and laboratory activities and compilation of research data were done by the corresponding author. Both authors thoroughly evaluated the research results, and performed the statistical analysis and literature search. Both authors produced the first draft of the manuscript which was thoroughly reviewed, corrected and improved by the author HD. Both authors read and approved the final version of the manuscript.

Research Article

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ABSTRACT

Field and laboratory experiments were conducted in the wheat growing belt of south-eastern Ethiopia to assess effects of rate and in-season N application on seed and seedling performance of local and improved bread wheat varieties. For the field experiments, a factorial combination of four N levels, two bread wheat varieties, and three times of N application were laid out in a Randomized Complete Block Design with three replicates. Laboratory tests were conducted in a Completely Randomized Design with four replicates to evaluate seed germination capacity and seedling vigor. The rate and timing of N application had significant ($P = .01$) effects on seed hectolitre weight, seed germination capacity and seedling vigor index. 1000-kernels weight was not affected by the rate of N application but significantly influenced by time of N application. Three times split application of N at 120 kg ha^{-1} resulted in significantly ($P = .01$) higher hectolitre weight, percentage of normal seedlings, seed germination speed, seedling dry weight and vigor index compared to the other treatments. The results revealed that application of 120 kg N ha^{-1} in three-split doses with ¼ dose at planting, ½ dose at mid-tillering and ¼ dose at anthesis led to enhanced seed quality and seedling performance of the crop.

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important world cereal crops and is a staple food for about one-third of the world's population [1]. In Ethiopia, it is grown annually on about 1.68 million ha of land with a total production of about 3.08 million t which makes the country the largest wheat producer in sub-Saharan Africa [2]. However, the average yield of wheat in the country is low (1.83 t ha^{-1}), which is only about half of the world average of 2.5 t ha^{-1}. The low crop productivity in the country may be attributed, among other factors, to low inherent soil fertility as well as meager and ill-timed N applications [3, 4]. Use of low rates of nitrogen (N) fertilizer is one of the major factors leading to low crop yields in developing countries [5]. Nitrogen deficiency has often constrained wheat production in wet areas [6, 7]. Availability of good quality seed is also an important factor limiting wheat productivity [8]. Application of N fertilizer resulted in a higher net assimilation rate [9], increased yield [10] and a higher seed protein content [11]. Timing of N application is also important for improving crop yields. Two to three equal split applications of N produced a higher grain yield of lowland rice compared to applying the whole dose of the nutrient at sowing [12]. The response of wheat to N fertilizer also varies with rate and timing of application relative to plant development [13].

Previous studies have revealed that seed quality is affected by N application with a profound influence on seed germination capacity [8, 14, and 15]. The amount and timing of N application had a significant effect on seed germination speed and early vigor, with the application of N at high levels (120 and 180 kg N ha^{-1}) just before anthesis resulting in a markedly increased seed germination capacity [8]. Thus, a well-balanced supply of N throughout the growing season of the crop is essential for seed production as it has an influence, not only on yield, but also on seed quality, which further affects seed germination capacity, seedling vigor, and the productivity of the succeeding crop.

In Ethiopia, wheat seed quality is low due to genetic and management factors [16]. Therefore, it is important to enhance seed quality of the crop targeting germination capacity and seedling vigor through proper plant nutrition. Little research has been done in the country to study the effects of N application on wheat seed planting values. Such a study is important to elucidate the influence of rate and timing of N application on seed quality of the crop. The results may lead to optimizing the quantity and timing of application of the nutrient for producing wheat seed with enhanced planting values for higher productivity of the crop. Therefore, this paper presents the results of a study conducted with the objective of evaluating the effect of N rate and timing of application on seed quality, seed germination capacity and seedling vigor of bread wheat.

2. MATERIALS AND METHODS

2.1 Field Experiment

2.1.1 Description of the experimental sites

Field experiments were conducted in two different environments in the Bale and West Arsi Zones of Ethiopia during the 2010 main cropping season. The experiments were carried out at Sinana (located at 7°7' N, 40°10' E) in Bale an d Adaba (located at 7º01'N, 39º24'E) in the

West Arsi Zones of Ethiopia. The two experimental sites have elevations of 2,400 and 2,300 m above sea level, respectively.

Sinana is characterized by a bi-modal rainfall pattern with two growing seasons. Annual rainfall, and mean minimum and maximum temperatures of the site during the experimental year were 1,464 mm, 10.1ºC, and 20.4ºC, respectively. On the other hand, Adaba is characterized by a mono-modal rainfall pattern, and received rainfall of 890.5 mm during the experimental year. The mean minimum and maximum temperatures at Adaba, during the cropping year were 8.15ºC and 23.82ºC, respectively.

Analysis of soil samples, before sowing, indicated that the soil texture of each experimental site was dominated by clay. Available Olsen P values were 13.3 ppm for Adaba and 15.4 ppm for Sinana. The available soil K content was 897.2 ppm at Adaba and 965.6 ppm at Sinana. Soil pH of the experimental sites was nearly neutral (6.9 at Adaba and 7.1 at Sinana). These values are in the pH range suitable for N availability. The soil of Adaba has an organic matter content of 2.2% whereas that of Sinana has 1.9%. The experimental soils had low levels of total N (0.11% and 0.14%, respectively, for Adaba and Sinana). According to Food and Agricultural Organization of the United Nations (FAO) [17], the P content of the soil is medium. The K content, at both sites, was very high [17,18]. The organic C and the total soil N contents of both sites were low [18]. The low organic matter and total N contents of the soils signify that the release of NO_3^{-1} from mineralization of organic matter [19] would be low and application of external sources of N is required to maximize crop yields.

2.1.2 Experimental materials

Nitrogen fertilizer in the form of urea (46% N) and phosphate fertilizer in the form of triple super phosphate (TSP) (20% P) were used as fertilizer material for the study. Two bread wheat varieties, namely, Madda Walabu (HAR-1480) and Hollandi (local) with different morphological characteristics and yield potential were used as test crops. Madda Walabu is a CIMMYT origin semi-dwarf improved wheat variety, which was released by Sinana Agricultural Research Centre (SARC) while Hollandi is a local variety and is used for crossing in SARC breeding programs for its merits (adaptability and bread-making quality). The improved variety, Madda Walabu, was chosen because it is high yielding, well adapted to the area, and is widely grown by smallholder farmers as well as large scale commercial wheat producers in the region.

2.1.3 Treatments and experimental design

The treatments consisted of four levels of N (30, 60, 90 and 120 kg N ha^{-1}), two bread wheat varieties (Madda Walabu and Holandi) and three times of N application (½ at sowing and ½ at mid-tillering; ¼ at sowing, ½ at mid-tillering and ¼ at anthesis; ⅓ at sowing, ⅓ at mid-tillering and ⅓ at anthesis). The experiment at each site was laid out in a randomized complete block design (RCBD) with a factorial arrangement of 4 x 2 x 3 = 24 treatment combinations and was replicated three times. The times of N application were adjusted according to Zadoks decimal growth stage for wheat at the time when moisture was available for nutrient dissolution and absorption.

2.2 Laboratory Experiments

2.2.1 Determination of seed germination capacity and vigor

After harvest standard germination tests for seeds obtained from control and treated plots were carried out between two layers of moist filter paper according to International Seed Testing Association [20] rules to evaluate treatment effects on seed germination capacity. The working samples, consisting of 400 pure seeds from each treatment, were counted using an electronic seed counter and tested in a factorial Completely Randomized Design (CRD) in four replicates of 100 seeds. The seeds were placed at uniform spacing between layers of filter paper moistened with distilled water and rolled as a substratum. The prepared rolls were placed in a slanting position in plastic containers and incubated in a growth chamber at the temperature of 20ºC and relative humidity of 80% under illumination. Samples were monitored daily and supplied with deionized water when needed. Seedling vigor was determined based on seedling dry weight, which is often measured as an indicator of seedling vigor. Seedling dry weight was measured after the final count of the standard germination test. Ten normal seedlings were randomly sampled from each replicate and dried in an oven at 60ºC to a constant weight.

2.2.2 Determination of seed germination speed

Seed germination speed, which also expresses seedling vigor and germination rate, was measured to determine treatment effects. For this purpose, another laboratory experiment was carried out using 100 seeds from each treatment. The seeds were sown on top of moist double filter paper arranged in a factorial CRD with four replicates of 25 seeds. The prepared samples were placed in Petri dishes and incubated at 20ºC in a growth chamber in the dark. Subsequently, seeds were evaluated for the presence of normal seedlings from the fourth to the eighth day of germination. Seedlings were counted and removed daily.

2.3 Data Measurement

Kernels weight (g): it was determined based on the weight of 1000 seeds sampled from the grain yields of each treatment through counting using an electric seed counter and weighing with an electronic balance.

Hectolitre Weight (Kg hL^{-1}): It was determined for dockage-free grain samples using Seedburo hectolitre mass device and electronic balance.

Germination capacity (%): on the final day of the test period, germination components were categorized into normal and abnormal seedlings, fresh ungerminated and dead seeds, according to international seed testing rules [20]. The percentage of each component was calculated based on the number of seeds used in the test.

Seedling dry weight (mg dry weight/germinal seedling): this was measured as the average oven dry weight of ten randomly selected seedlings by weighing on an electric balance. *Seed germination speed*: this was calculated using the following formula [21].

$$Germination\ speed = \frac{\sum n}{\sum (n \times Dn)} \times 100$$

Where, n = number of normal seedlings germinated on day (D) and Dn = number of days from sowing corresponding to n.

Seedling vigor index: it was calculated by multiplying the standard germination percentage by mean seedling dry weight [22].

$$Seedling\ vigor\ index = Seedling\ dry\ weight\ x\ germination\ percentage$$

2.4 Statistical Analysis

The data were subjected to analyses of variance using the SAS GLM procedure. Bartlett's homogeneity test of error variance was performed for all data of the two environments using Minitab software before performing a combined analysis. For seed germination components, variance analysis was done on the data transformed by the following function. $y = \arcsin\sqrt{p/100}$ where p is the percentage of the germination component. Significant differences among treatments were computed using the Duncan's Multiple Range Tests.

3. RESULTS AND DISCUSSION

All measured and analyzed data were subjected to a homogeneity test of error variances using Bartlett's $\chi 2$ test. Homogeneity of error variances was observed for the two test environments for all data. Hence, the results of the combined analysis for the homogenous data are presented and discussed.

3.1 Kernel Weight and Hectolitre Weight

Analysis of variance for thousand kernel weight indicated no significant effect of N application rate and variety interactions with both N rate and time of applications. However, there were significant influences of time of N application and variety on the trait. Hectolitre weight (HLW) was significantly (*P = .05*) affected by N rate, time of N application, and variety. However, variety interactions with both N rate and time of applications were non-significant indicating similar performances of the varieties in HLW under different N supply (Table 1).

Mean 1000-kernel weight of the varieties averaged over all N rate and time of application indicated that Madda Walabu produced significantly heavier kernels which was about 20.8% higher than the kernel weight of Hollandi. The result might indicate that kernel weight was less likely to be affected by the amount of N supply compared to the genetic effect. The result is in agreement with other findings [23] in which non-significant effect of N application rate on kernel weight was reported. However, it contradicts with the findings of other studies conducted in Ethiopia [24] in which a significant effect of N levels on 1000-kernel weight was reported. The three-time split N application of ¼ at planting, ½ at mid-tillering and ¼ at anthesis (¼P, ½MT and ¼A) produced heavier 1000-kernel weight (37.13 g). The three-time equal split N application at planting, mid-tillering and anthesis (⅓P, ⅓MT and ⅓A) led to the production of an intermediate 1000-kernel weight, which was in statistical parity with the 1000-kernel weights obtained in response to the other timings of N application. Compared to the three-time N application of ¼P, ½MT and ¼A, the two-time equal split application at planting and mid-tillering (½P and ½MT) resulted in the production of seeds with 4.1% lower 1000-kernel weight (Table 1).

Hectolitre weight (HLW) was observed to increase with the increasing N application rate in which the lowest (68.43 kg hL^{-1}) and highest (72.69 kg hL^{-1}) values were obtained, respectively, at the application rates of 30 and 120 kg N ha^{-1}. Compared to the lower N application rates of 30 and 60 kg ha^{-1}, 120 kg N ha^{-1} resulted in 5.9% and 4.7% higher HLW, respectively. Comparing the two varieties, across the locations and N applications, the highest HLW (78.49 kg hL^{-1}) was obtained for Madda Walabu which was 20.6% higher than the weight obtained for Hollandi. Other studies conducted in Chile [25] also indicated that HLW in bread wheat was positively affected by higher N application rate of up to 300 kg N ha^{-1} in addition to strong influences of the genetic background of cultivars. But, the result contradicts with the findings of other scholars [26] who reported a significantly declining HLW with increasing N fertilizer rate.

The three-time split N application of ¼P, ½MT and ¼A also resulted in higher HLW (71.49 kg hL^{-1}), which was in statistical parity with the HLW obtained in response to three-time application of ⅓P, ⅓MT and ⅓A. It was found that the two-time N application of ½P and ½MT produced lower HLW which was comparable with the three-time equal split application at planting, mid-tillering and anthesis. The result might indicate the importance of HLW as a seed physical quality trait more than 1000-kernel weight which was more affected by N supply. In other reports [27] it was indicated that three-split N applications at planting, tillering, and stem elongation failed to increase wheat HLW.

Table 1. Thousand kernel weight and hectolitre weight of bread wheat as influenced by N rate and time of application

	Thousand kernels weight (g)			**Hectolitre weight (hL^{-1})**		
Variety (V)	**Madda Walabu**	**Hollandi**	**Mean**	**Madda Walabu**	**Hollandi**	**Mean**
N rate (kg ha^{-1}) (NR)						
30	39.99	30.85	35.42	76.76	60.10	68.43^{c}
60	40.29	31.87	36.08	77.67	60.80	69.24^{bc}
90	40.75	32.52	36.64	79.29	63.15	71.22^{ab}
120	40.95	32.94	36.94	80.26	65.11	72.69^{a}
	V	NR	V x NR	V	NR	V x NR
SE ±	0.32	0.46	0.65	0.57	0.81	1.15
CD	0.92	NS	NS	1.64	2.31	NS
N application time (NT)						
½P and ½MT	39.87	31.36	35.62^{b}	76.61	61.32	68.97^{b}
¼P, ½MT and ¼A	41.33	32.93	37.13^{a}	80.16	62.82	71.49^{a}
⅓P, ⅓MT and ⅓A	40.28	31.85	36.06^{ab}	78.70	62.73	70.72^{ab}
Mean	40.49^{a}	32.05^{b}		78.49^{a}	62.29^{b}	
	V	NT	V x NT	V	NT	V x NT
SE ±	0.32	0.40	0.56	0.57	0.70	0.99
CD	0.92	1.13	NS	1.64	2.00	NS
CV (%)	5.36			4.89		

Where, P, MT and A indicate N application at planting, mid-tillering and anthesis, respectively; NS = non-significant. Variable means followed by the same letters are not significantly different (P = .05) according to the Duncan's Multiple Range Tests.

3.2 Seed Germination Capacity

The laboratory experiment showed normal germination of seed samples obtained from each treatment i.e. initially no seed germination was observed from the start of incubation until the 3rd day. Small numbers of seeds were germinated on the 4th day of the incubation period. A rapid increase in the number of germinating seeds was observed on the 5th and 6th days. This was followed by a decrease in the appearance of newly germinating seeds after the 7th day of incubation (Fig. 1). Application of the highest N rate (120 kg N ha^{-1}) resulted in a significantly higher percentage of the total number of normal seedlings and proportion of germinating seeds in the early incubation period (4th to 5th days) for variety Madda Walabu. However, for the local variety, the proportion of germinating seeds in the same period did not differ among treatments. For the local variety, application of 120 kg N ha^{-1} resulted in the highest proportion of germinated seeds but on the 6th day of incubation (Fig. 2). Generally, the percentage of seed that germinated during the incubation period more or less followed a normal distribution curve that predicts the time to seedling emergence under different N application treatments.

Analysis of variance for the data obtained at the end of the incubation period, revealed a significant influence of N rate and time of N application on the percentages of normal and abnormal seedlings, and dead seeds. The analysis showed there was no effect of time of N application on the proportion of fresh ungerminated seeds. Across all N rates and times of application, however, the varieties differed significantly ($P = .01$) in terms of the percentage of normal and abnormal seedlings. However, the varieties did not interact with N rate and time of application to influence all the germination components.

The highest percentage of normal seedlings (93.3%) was obtained in response to the application of 120 kg N ha^{-1} and the lowest percentage (88.6%) was obtained at 30 kg N ha^{-1}. Conversely, the highest percentage of abnormal seedlings (5.8%) was observed at the lowest N application rate and the lowest (3.6%) was obtained at the highest N application rate. The percentage of dead and fresh ungerminated seed decreased significantly in response to increasing the rate of N application. Averaged over all N supply treatments, the variety Madda Walabu produced a higher percentage of normal (92.2%) and a lower percentage of abnormal (4.1%) seedlings. This indicates the superiority of the improved wheat variety over the local variety in terms of germination capacity.

The three-time split N applications resulted in the highest percentages of normal seedlings while the two-time split N application produced the lowest percentage. These results are consistent with other findings [8, 15] in which significantly higher percentage of normal seedlings were reported in response to application of higher rates of N. The higher percentage of normal and lower percentage of abnormal seedlings in response to the application of the highest N rate in the three splits might reflect the significant role played by N in enhancing assimilate accumulation in the seeds, which may have promoted seedling growth in this study. These results evidently accentuate the importance of N for improved wheat seed germination, and hence improved crop productivity.

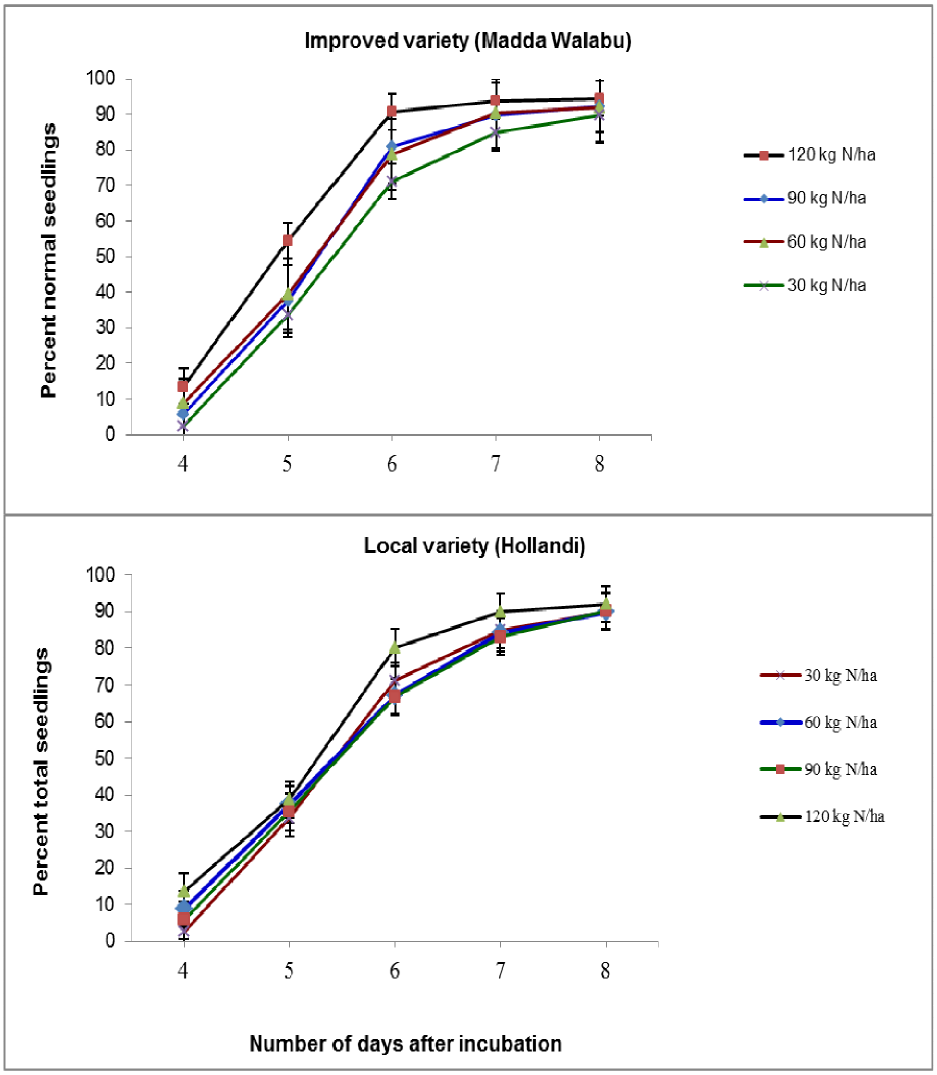

Fig. 1. Effect of N application rate on germination of normal seedlings of improved and local bread wheat varieties

The varieties significantly differed in the percentages of fresh ungerminated seeds and dead seeds, in which variety Madda Walabu had the lowest proportions. The result indicates the importance of field application of higher N fertilizer rates on a wheat crop to minimize the production of non-germinating seeds. Seeds produced in response to the two-time N application of ½P and ½MT had 28.6% higher percentage of dead seeds than seeds produced with three-time split applications. The superiority of the three-time split N application in lowering the number of dead seeds may be attributed to the importance of applying N at the three critical growth stages for the synthesis and accumulation of assimilate in seed during grain filling that promoted not only seed weight, as shown in the preceding sections, but also seed planting values.

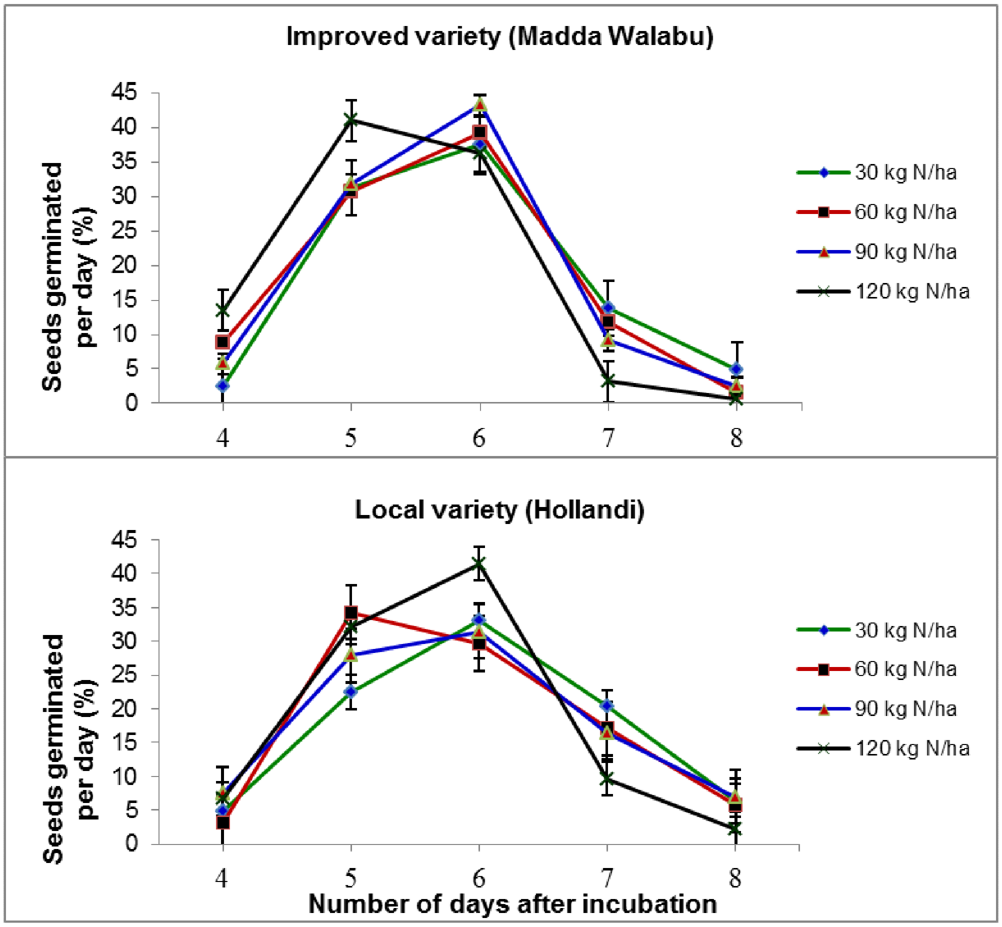

Fig. 2. Effect of N application rate on germination of normal seedlings per day of improved and local bread wheat varieties

The results of this experiment clearly indicate that the capacity of seed germination is closely associated with N supply. The results also signify that the variety with higher N response have higher germination capacity. Other authors also suggested that, among various agro-management practices which are used to raise successful plants, nutrient supply seems more promising to enhance the capacity of germination [28]. In another report, it was revealed that the higher germination capacity of seeds with higher N content was associated with a higher metabolic process which led to more rapid rate of germination [29].

3.3 Seed Germination Speed, Seedling Dry Weight and Vigor Index

Analysis of variance indicated a significant (P = .01) effect of field N application and timing on seed germination speed, seedling dry weight, and seedling vigor index. There were also significant variations between the varieties for the measured parameters. However, the varieties did not interact with N rate and time of application. Seed germination calculated from the 4th to the 8th (final) days of the standard germination test revealed higher speed of seed obtained from plants supplied with 120 kg N ha^{-1}. Field application of 30 kg N ha^{-1}, on the other hand, resulted in a significantly lower seed germination speed (Fig. 3). Three-time

N application of ¼P, ½MT and ¼A resulted in a significantly higher speed of seed germination.

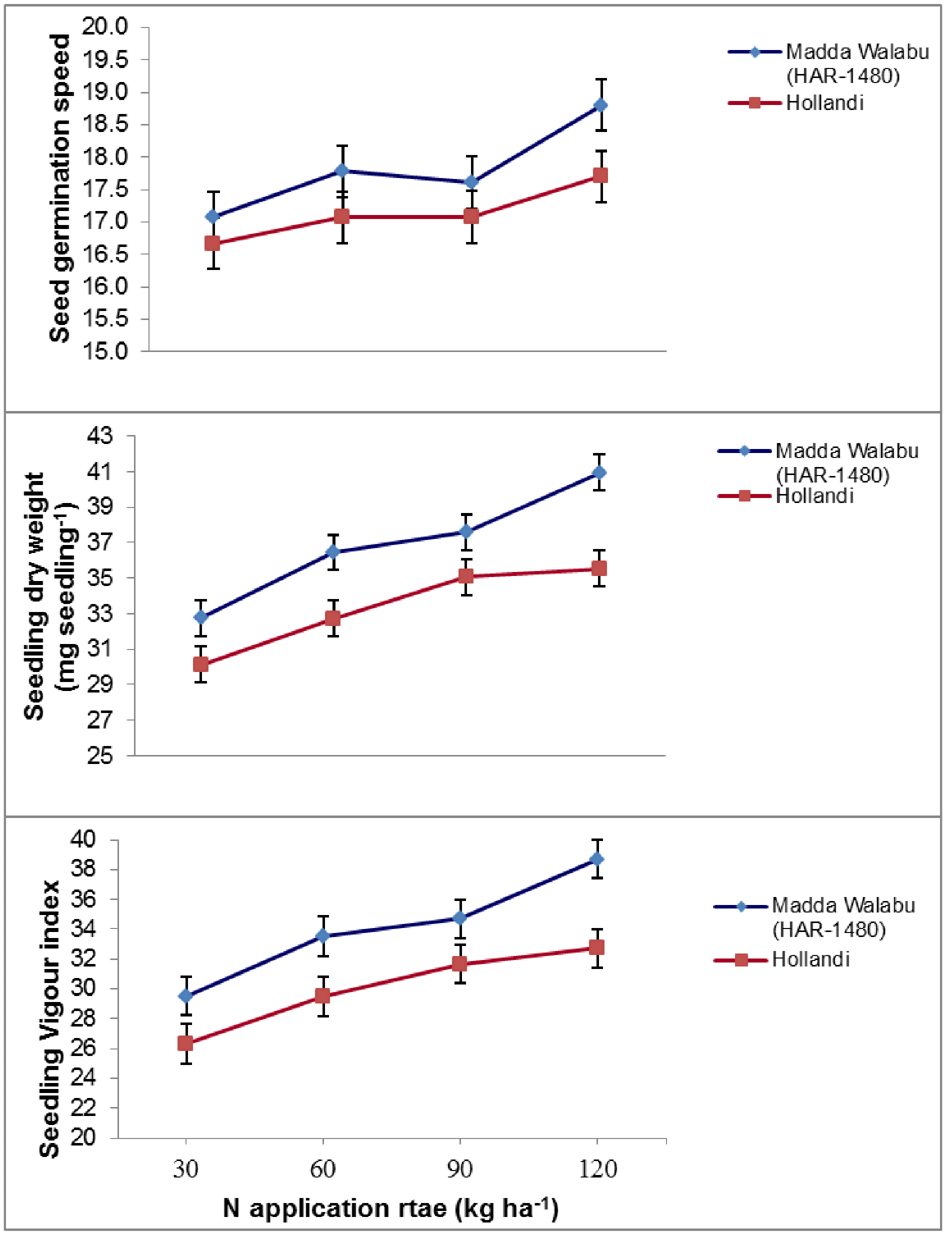

Fig. 3. Seed germination speed, seedling dry weight and vigor index of bread wheat seeds obtained from crop supplied with different N rates

Nitrogen application at 120 kg N ha^{-1} also resulted in higher germinal seedling dry weight (38.2 mg seedling^{-1}). Each increase in N application resulted in a significant increase in germinal seedling dry weight. Mean comparison of the varieties indicated a higher seedling dry weight of Madda Walabu compared to Hollandi, which was higher by 9.7%. Seeds obtained in response to the application of N in two equal splits at sowing and mid-tillering resulted in lower seedling dry weights compared to the three-time N application treatments (Fig. 4).

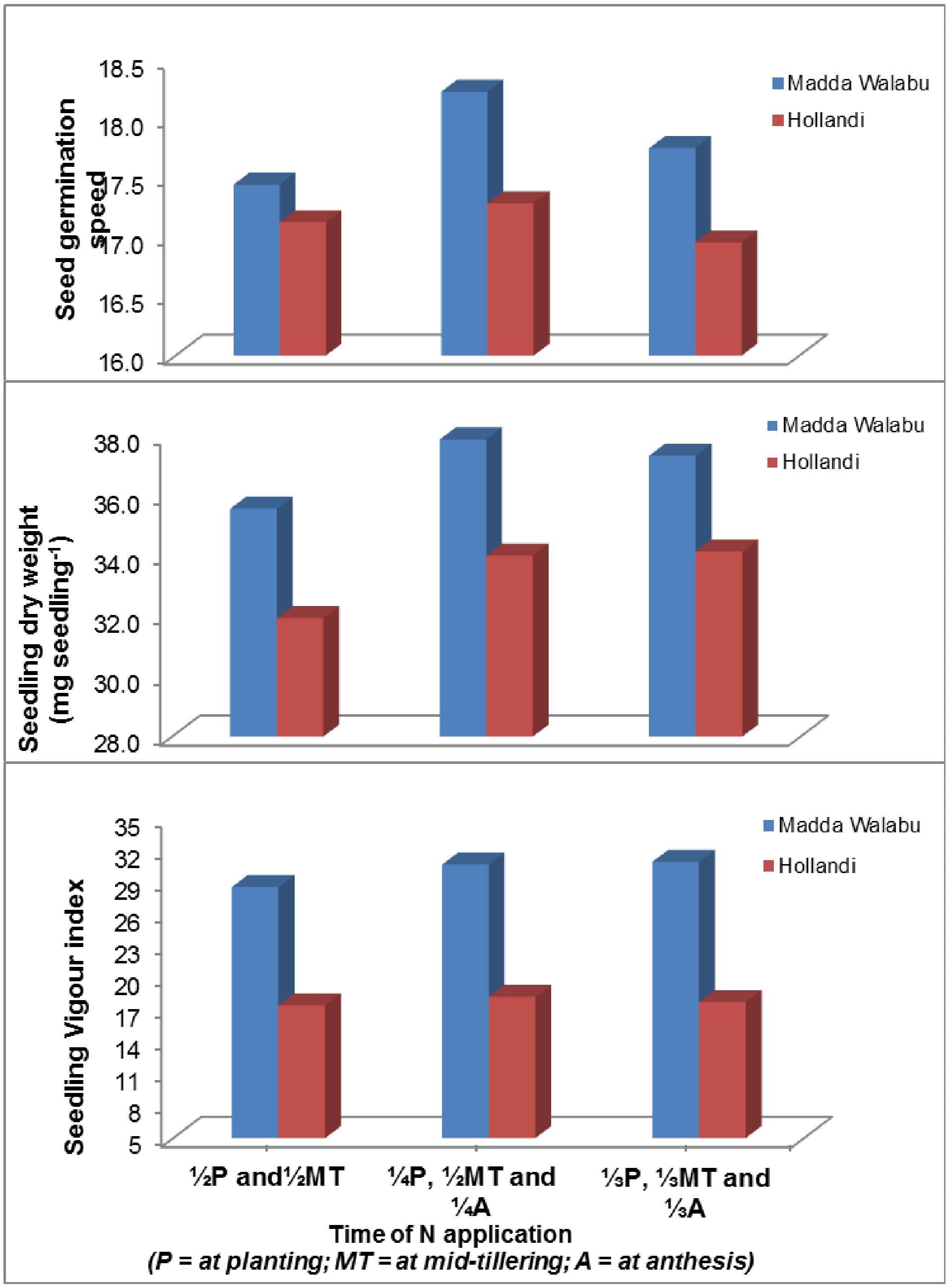

Fig. 4. Seed germination speed, seedling dry weight, and vigor index of bread wheat seeds obtained from crop supplied with N at different growth stages

In another finding, a positive association was reported between seed nutrient content and seedling dry weight [30]. The authors found that seedlings developed from seeds with high N contents showed increased water absorption and oxygen consumption during germination, and produced heavier seedlings. Corroborating the current results, maximum seedling dry weight was recorded from seed samples obtained from plants supplied with N at sowing and tillering, and from plants treated with foliar application of N at post anthesis.

Seedling vigor index was also significantly increased with increased rates of N application in which the highest (35.7) and the lowest (27.9) indices were obtained at 120 and 30 kg N ha^{-1}, respectively (Fig. 3). The difference between the varieties in vigor index was 11.9% higher for the improved variety. The vigor index of seed produced by plants treated with ½P and ½MT was significantly lower than the vigor index of seeds produced by plants treated with three-time split N applications (Fig. 4).

The higher germination speed, seedling dry weight, and vigor index of seed from plants supplied with higher N rates and three times split application may be ascribed to increased assimilate synthesis and translocation to the grain which may have resulted in higher contents of protein and other food reserves. Corroborating these results, other authors [8, 15] also reported vigorous seedling growth for seeds obtained from wheat plants supplied with high N rates.

4. CONCLUSION

Low available soil N and low rates as well as ill-timing of N application are major factors constraining quality of seed and planting values of seed produced in major wheat growing areas of Ethiopia. The results of this study indicate that the hectolitre weight, capacity of seed germination, and seedling growth were significantly enhanced in response to increased application of N in three split doses at the three distinct growth stages of a wheat crop. Higher N rate and three split applications resulted in significantly higher HLW. The varieties highly differed in their physical seed quality characteristics in which the improved variety produced higher TKW and HLW. The highest values for seed germination speed, seedling dry weight, and vigor index were obtained in response to the application of 120 kg N ha^{-1}. In-season N applications of ¼P, ½MT and ¼A and ⅓P, ⅓MT and ⅓A also resulted in the highest seed germination capacity and seedling vigor. The improved wheat variety had higher performances for these variables than the local variety. It could, therefore, be concluded that application of 120 kg N ha^{-1} in three splits at sowing, mid-tillering and anthesis resulted in optimum seed germination capacity and seedling performance of the variety in the study areas. Therefore, farmers should cultivate the improved Madda Walabu variety by applying 120 kg N ha^{-1} in three splits during the three distinct growth stages of the plant for optimizing the planting values of seed produced and productivity of the crop in the region.

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COMPETING INTERESTS

We, the authors, declare that there is no competing interest from other people or organizations which inappropriately influences this work.

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