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Effect of nitrogen stress amelioration on status of break flour yield of soft wheat for making bread

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the largest cereal source of protein (20%) and the second largest source of calories (21%) worldwide. Nitrogen is a major factor that affects bread wheat production in most soils in Uganda. The objective of this study was to determine the effect of nitrogen stress amelioration on break flour yield. A field study was conducted at Buginyanya Zonal Agricultural Research and Development Institute (BugiZARDI) in the highlands of Eastern Uganda, during 2013-2014. Treatments included 0, 30, 60 and 90 Kg N ha^{-1} with UW400 as test genotype. Site soil N was 0.15%, way below the critical limit of 0.25%. Maximum break flour yield was obtained at 60 kg N ha^{-1} and the least at 0 kg N ha^{-1}. There was a direct negative effect of Nitrogen on the level of grain starch; while no significant effect was displayed between Nitrogen application rates and crude protein levels. A weak negative, but significant ($P < 0.05$) correlation accrued between grain yield and BFY (r=-0.3); while the relationship between starch and crude protein was strong, though equally negative (r=-0.9). The agronomic nitrogen rate required ameliorating nitrogen stress to obtain high break flour and grain yield is 60 kg ha^{-1}.

Keywords: Crude protein, starch, *Triticum aestivum*, Uganda, UW400
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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the largest cereal source of protein (20%) and the second largest cereal source of calories (21%) worldwide (Paroda et al., 2013), largely consumed as bread. Among the key considerations of the bread industry is break flour, which is one of the parameters that describe milling quality of wheat (starch damage, milling yield, flour protein). Break flour is defined as the total weight of flour obtained from the break rollers on an industrial machine, during the milling process (Miles, 2010). There is scanty information on the relationship between plant nutrition and break flour yield and quality, the understanding of which may be necessary to enhance bread production levels and the overall returns to fertilizer inputs (Edmund et al., 2008).
Among the candidate nutrients with the potential to influence the construction of break flour is nitrogen. Nitrogen, through its protein derivatives, is important in sustaining the nature of wheat break flour particles during the milling process, into fine white flour blended for premium bread products (Malik, 2009). On the other hand, excess nitrogen in the soil may result in an imbalance in protein to starch ratio, perhaps exceeding

the minimum required for making bread. This imbalance may result into flour with high gluten and low damaged starch, which affects the structure and texture of bread from wheat break flour (Shewry, 2006). Therefore, in order to achieve quality bread from a wheat fertilization program, attention needs to be traced right from the status of nitrogen in the soil of the field, through crop yield, up to the physio-chemical integrity of the wheat grain destined for bread production.

Although Nuttall et al. (2017) have shade light on the relationship between N levels in the soil, grain size and shape of bread wheat grains, the link between N nutrition especially N stress amelioration and wheat break flour yield and quality, remain to be investigated. The objective of this study, therefore, was to determine the effect of N stress amelioration through fertilizer application on wheat break flour yield of a highland soft wheat genotype.

MATERIALS AND METHODS

Study site

This study was conducted at Buginyanya Zonal Agricultural Research and Development Institute (BugiZARDI), in the highlands of Eastern Uganda, during two rainy seasons (2013b and 2014a). The experimental site was located at N01'1643.7, E034'22303, at an altitude of 1902 meters above sea level (masl). The area experiences a bimodal rainfall pattern, with a mean amount of rainfall of 2549 mm yr^{-1}, mean maximum temperature of 21ºC and relative humidity of 64% (Meteorological Department, BugiZARDI, 2014). Site soil texture was sandy clay loam, and was previously classified as Andosols (Takahashi and Shoji, 2002).

Treatments and research design

Treatments include; N applied at 0, 30, 60 and 90 kg ha^{-1} applied in the form of urea (46 % N). Urea was applied in two splits, one split (50%) at planting, and the second split at about 28 days prior to stem elongation (Pask, 2012). This was done to minimize excessive leaching before plant establishment in the field. The fertilizer was spread within shallow furrows, dug approximately 5 cm away from the seeded furrows. The furrows were closed manually, immediately after fertilizer application to prevent fertilizer volatilization losses. Treatments were laid out in Randomized Complete Block Design (RCBD), replicated three times.

Test wheat genotype

The material of study was UW400, an elite line which was identified in 2003 and was being grown by some farmers from eastern Uganda who participated in adaptive trials that led to its selection. It is semi-dwarf and matures in 85-95 days. It has good resistance to yellow rust, leaf rust and stem rust (Ug99), and is recommended for the high altitude (1800-3000 masl) areas of Uganda (Wagoire and Wasukira, 2012, unpublished).

Field management

Plot size was 5 m by 3 m, with one meters separation between blocks. Seeds were sown in furrows continuously with an inter-row spacing of 0.3 m. Weeding was done at 3 weeks (stem elongation stage) and 6 weeks (booting stage) after sowing, using a hand hoe. Off-types (defective plants) that appeared in some plots were removed manually, to maintain field uniformity.

Data collection and analysis

Pre-experimental site soil analysis

Composite soil samples were taken from the study site, at 0-15cm depth, prior to establishing the experiment. The samples were air-dried for 7 days and then pounded using a porcelain mortar and pestle, before being sieved through 2 mm sieves. The ground samples were used for baseline analysis for soil pH (in water), total N and organic matter, and exchangeable potassium, using routine procedures (Okalebo et al., 2002). The test values were compared with critical values according to Anderson and Ingram (1993).

Grain yield

Wheat kernels were harvested at physiological maturity, determined by attainment of grain hardness that could not be dented by a thumbnail (Pask, 2012). At this stage, all the glumes were dry. Kernel yield was recorded for nine rows (harvest area = 12 m^2), out of eleven rows in each plot. This was approximately 40 plants. The harvested kernels from each plot were weighed using a spring balance. The kernels were sun-dried for 5 days to achieve a moisture content of approximately 12%. The grain yield recorded was extrapolated from 12 to 10,000 m^2 to obtain grain yield in kg per ha.

Break flour yield

Break flour yield was determined using a Chopin laboratory Mill CD1 auto, following principles of Chopin Technologies, 2013 at the Barkhresa Grain Milling Company in Kampala, Uganda. The procedure included the introduction of 2 kg of grain into the feed hopper for onward delivery into the grinding system. The grain was

Table 1. Pre-experimental site soil analysis results.

Soil property	Test values	Critical values†
pH (in water)	5.95	5.50
Total N (%)	0.15	0.25
Organic carbon (%)	3.81	3.00
Exch. K^+ (cmol. kg^{-1})	0.78	0.33

† Minimum values according to Anderson and Ingram (1993), N - Nitrogen, K^+- Potassium.

ground by three grooved cylinders through two successive grindings. The ground samples from the break rolls were sieved through a breaking sieve (0.5 µm) to the break container. The resulting flour was weighed using a laboratory digital balance, and then used to determine break flour yield (Equation 1).

$$\%\text{ Break flour yield} = \frac{\text{Weight of flour from break rolls}}{2\text{ kg of wheat grain}} \times 100 \quad \ldots\ldots\ldots\ 1$$

Crude protein content

The crude protein content of wheat flour samples obtained from the break rolls was analyzed using the Kjeldal technique, using the wet oxidation procedure and crude protein calculated as follow:

$$\%\text{ Crude Protein} = \%\text{ N} \times 5.7 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ 2$$

Where: N = % nitrogen, and 5.7 = wheat flour conversion factor accounting for amino acid composition and non-protein nitrogen (Edmund et al., 2008).

Starch content

Starch was extracted from the ground samples using perchloric acid, and subsequently reacted with the anthrone reagent (Bartkiene, 2012). The resulting colour was measured using a photo spectrometer at 630 nm and starch calculated on dry matter basis as follows:

$$\%\text{ Starch} = \frac{22.5 \times \text{O.D of sample}}{\text{Weight of sample} \times \text{O.D of } 100\ \mu\text{g standard of glucose}} \quad \ldots\ldots\ldots\ldots\ 3$$

Where: O.D = Optical density (absorbance) and 22.5 = glucose concentration (Bartkiene, 2012)

Statistical analysis

The data were entered into Microsoft Excel, tested for normality using the W-test and subjected it to the analysis of variance (ANOVA) using GenStat 12th version for Windows. Significant means were separated using the Least Significance Difference (LSD) at 5% probability level. Correlation analysis was done for break flour yield and quality parameters (crude protein and starch).

RESULTS

Pre-experimental site soil characteristics

Results for pre-experiment site soil analysis are presented in Table 1. It is evident that the level of total N was stressful for viable production of wheat; despite the fact that organic carbon superseded its critical value. Soil pH and exchangeable potassium (K^+) were generally adequate for proper wheat production.

Grain yield

Application of N significantly ($P < 0.05$) increased bread wheat grain yield (Figure 1) climaxed with a yield gain of up to 71.9 % at the nitrogen rate of 60 kg ha^{-1}. Beyond this point, the response became negative.

Break flour yield

Application of N caused a significant ($P<0.05$) effect on break flour yield (Figure 2). Break flour yield peaked with a gain of 41.3% at N rate of 60 kg ha^{-1}; before it subsequently declined.

Crude protein and grain starch

Application of N caused no significant effect ($P>0.05$) on crude protein (CP), although there were changes in CP at different rates of N. However, the effect of N application was significant ($P<0.05$), but depressed grain starch concentration. The depressions of grain starch were in the order of 2.48, 15.6 and 17.7 % for N rates at 30, 60 and 90 kg ha^{-1}, respectively (Table 2). The crude protein to starch ratio had no pattern among the different rates of N, though the highest ratio was obtained at 60 Kg ha^{-1}.

Parameter correlations

There was a negative, but significant ($P < 0.05$)

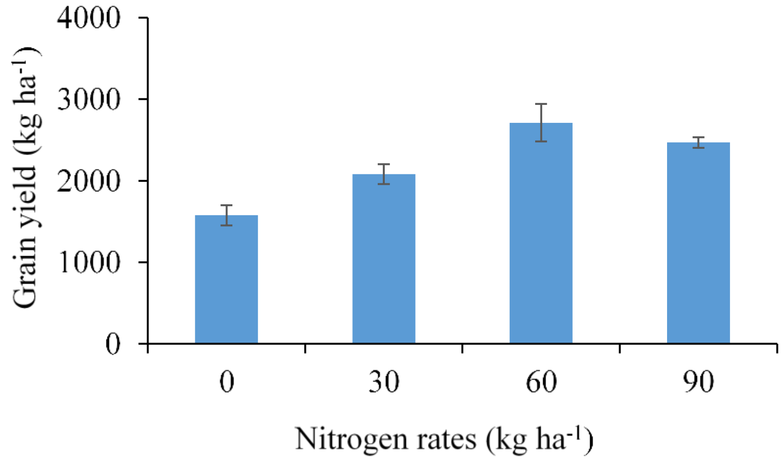

Figure 1. Effect of nitrogen on grain yield of UW400 bread wheat genotype in the highlands of eastern Uganda.

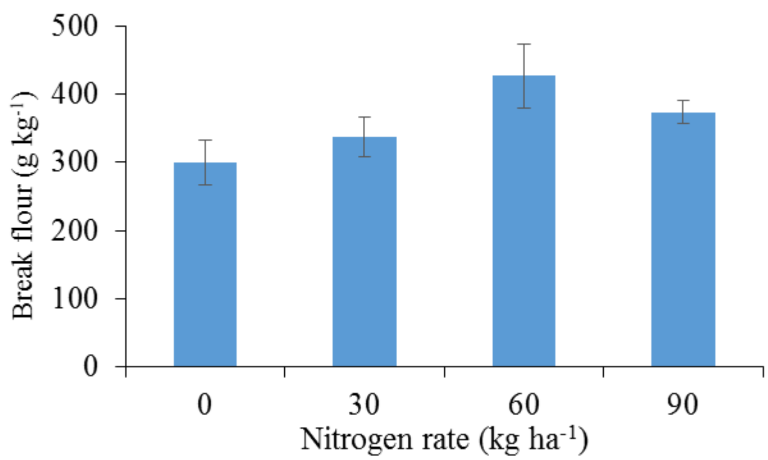

Figure 2. Effect of nitrogen on break flour yield of UW400 bread wheat genotype in the highlands of eastern Uganda.

correlation between grain yield and break flour yield (Figure 3); and an inverse but strong relationship between starch and crude protein (Figure 4). Similarly, there was a strong negative correlation between grain yield and starch (Figure 5).

DISCUSSION

Pre-experiment site soil analysis

Total N stress in the study site soil (Table 1) justifies the need for application of N to boost the productivity of wheat in this region. Wheat is renowned for requirement of substantial quantities of nitrogen throughout its growth cycle (Tamang et al., 2017). The presence of organic carbon at a level superseding the critical value (Table 1) seems to contradict the traditional belief that the level of soil organic carbon is positively correlated with total N content of a given soil (Xu et al., 2013). This could be attributed to the possibility that the organic matter fraction comprises of recalcitrant organic forms of lignin and polyphenols, which are known to be the ultimate residues of extreme organic matter decomposition, particularly in highly impoverished soils of the humid tropics (Yoneyana et al., 2015), such organic carbon is devoid of labile N fractions, though they contribute to quality of the overall productivity of the soil.

Grain yield

The positive response of bread wheat grain yield to N application (Figure 1) is typical of the wheat family which is heavy feeders of N, especially when high grain yield

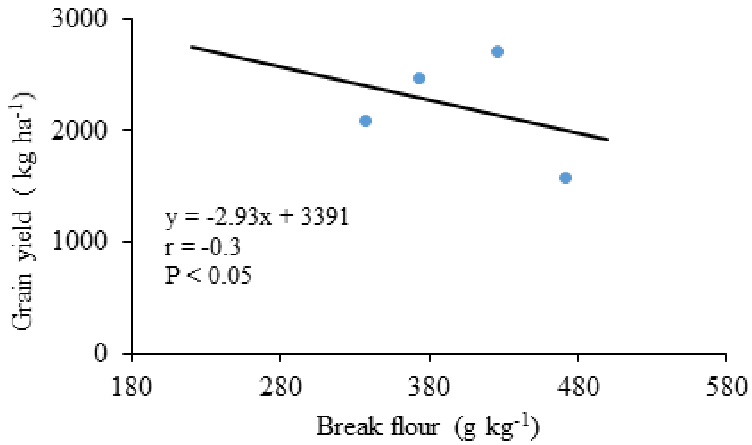

Figure 3. Correlation of grain yield and break flour of UW400 bread wheat genotype grown in the highlands of eastern Uganda.

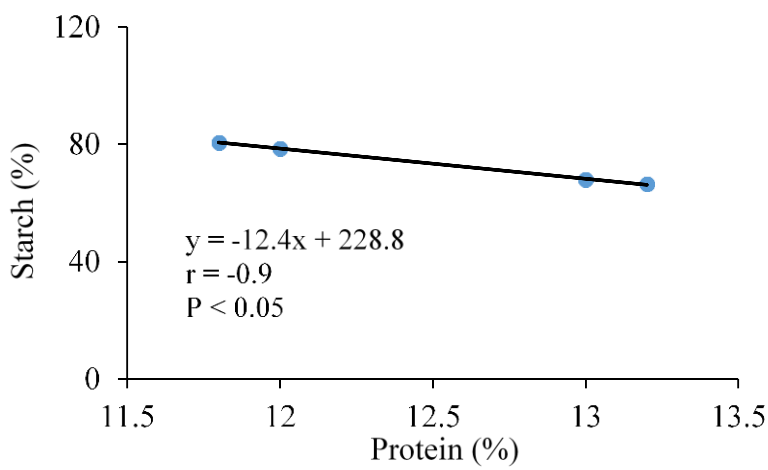

Figure 4. Correlation of starch and crude protein of UW400 bread wheat genotype grown in the highlands of eastern Uganda.

goals are targeted (Cowger et al., 2014). Ali et al. (2011) observed a positive grain yield response (58 %) of bread wheat, while working at the University College of Agriculture Sargodha in Pakistan. Grain yield response to N application in the present study, climaxed with a yield gain of up to 71.9% at the N rate of 60 kg ha^{-1}. The negative response to N application beyond the 60 kg ha^{-1} N rate could be due to the incursion of limitation of other nutrients such as available P, which was not taken care of in the soil fertility management regime. It could also be due to a buildup of salt effect electroconductivity which was not monitored. Therefore, for this variety of bread wheat (UW400), the N rate required to obtain grain agronomic maximum yield is 60 kg ha^{-1}. This contrasts with the rate of 90 kg ha^{-1} often used by farmers based on spillover, unscreened farmer to farmer information likely obtained from across the Kenya-Uganda border, where fertilizer smuggling is rampant (Kapchanga, 2008). Although there is still need for economic analysis in order to attain the farmer-desired N rate for use in the field, this study sets a benchmark for extension trials, particularly in the advent of the implementation of the national fertilizer strategy, whose program is currently being rolled out by the Ministry of

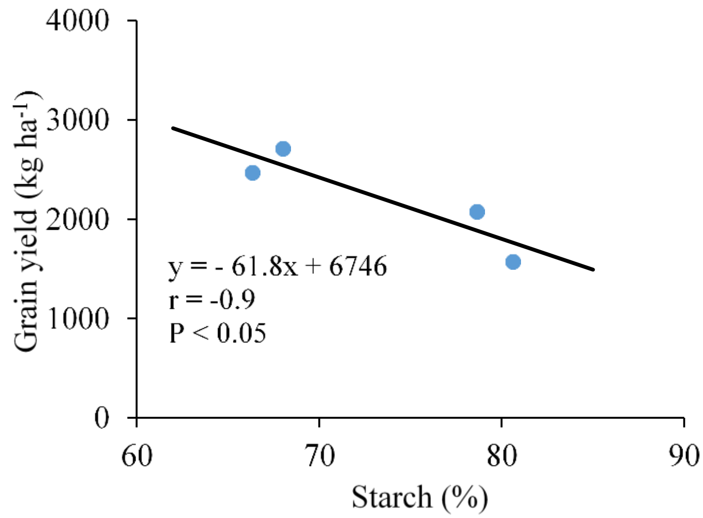

Figure 5. Correlation of grain yield and starch of UW400 bread wheat genotype grown in the highlands of eastern Uganda.

Table 2. Starch content and crude protein of UW400 bread wheat at different rates of nitrogen in the highlands of eastern Uganda.

Nitrogen rate (kg ha^{-1})	**Starch (g kg^{-1})**	**Crude protein (mg kg^{-1})**	**Crude protein : starch ratio**
0	806	130.7	0.162
30	786	117.4	0.149
60	680	133.3	0.196
90	663	127.8	0.192
LSD (<0.05)	9.7	NS	
CV%	11.1	11.5	

Agriculture, Animal Industry and Fisheries (MAAIF, 2016).

Break flour yield

The significant effect of N application on break flour yield (Figure 2) demonstrates the need for N application in the region to attain bread quality preferred by premium market consumers. An earlier study by Kimball et al. (2001), in the USA, revealed that break flour yield was an exceptional indicator of wheat flour particle size and relative hardness.

Based on the present study, the N rate required to obtain maximum break flour yield (21.3%) was 60 kg ha^{-1}. The percentage increase in break flour yield was lower than that of grain yield (Figure 2) at the same N rate, because break flour is a fraction of the total flour obtained from grain after milling. The apparent scarcity of literature on break flour's physio-chemical phenomena, in relation to plant nutrition in general, and N in particular, hinders elaborate discussions of relevant research findings to meaningful levels. The present study marks the beginning of investigations on bread wheat quality improvement, a subject hitherto overlooked in the mainstream research.

Crude protein

The non-significant effect of N on crude protein (Table 2) in bread wheat in this study may be attributed to fairly limited N applied. Protein is a major determinant of baking quality for bread and has been variously reported to have a positive significant response to nitrogen. Gerba et al. (2013) noted that nitrogen is required in larger quantities to significantly affect protein formation process. The most important protein for bread making is gluten, which comprises of monomeric gliadins and polymeric glutenins. Glutenins are primarily responsible for dough elasticity (strength) and gliadins for dough viscosity and extensibility (Foulkes et al., 2009). This study focused on CP per *se*, thus there is a need for further investigation on wheat gluten response levels to applied nitrogen.

Grain starch

The negative effect of N rate on grain starch production (Table 2) in bread wheat is contradictory to what appears in most literature sources (Aliu et al., 2012). This is so because starch is known to account for the bulk of the dry matter yield, which was registered in grain yield (Table 2). This contradiction can be explained by differences in the relative proportions of amylose and amylopectin caused by the rise in nitrogen rates; and more specifically the possibility of differences in densities of the molecular weights constituting these starch units. Xiong et al. (2014), in a study on the effect of N on cv. Xumai 30, hard red winter wheat observed a significant effect (P<0.05) of N rate on the distribution of endosperm starch granules in modified aleurone, different from the other subaleurone of dorsal, center of dorsal, subaleurone of ventral and center of ventral endosperm. Their effort however, did not link this starch behavior with the quality of wheat required for attaining premium bread properties. In our study, it might be true that starch levels were responding positively like grain yield in the traditional patterns (*Zhao et al.,* 2008), but the starch unit accumulated as a result of N application may be of a nature of low density thus resulting in a falsehood decline in overall starch yield.

The present study has demonstrated that N application causes a crude protein to starch ratio of 0.149-0.192. Protein to starch ratio is an aspect glaringly absent in bread wheat literature. Therefore, the implication of this observation, on the integrity of break flour in bread wheat remains a subject for investigation.

Parameter correlation

The slight negative correlation (r = -0.3) between grain yield and break flour yield (Figure 3) shows that increase in grain yield is weakly related to break flour yield. Literature on the relationship between grain yield and break flour yield is unavailable. However; this negative relationship may be attributed to increased nitrogen application which increases grain yield, with potential negative implication on a thousand grain weights, grain size and test weight; which are determinants for break flour yield and end-use quality for bread making (Abdipour et al., 2016).

There was a strong, though negative correlation (r = -0.9) between starch and crude protein (Figure 4). This observation is similar to what was observed by Kindred et al. (2008), while working on winter wheat in the UK whereby strong negative correlations were obtained. The strong negative correlation of starch to crude protein is attributed to the dilution effect; whereby high yielding varieties, nitrogen is distributed in a greater number of grains or within larger grains, thus lowering protein content. This dilution effect has been reported on from different wheat studies by Whitefield and Smith (1992) and Simmonds, (1995). From this study, the UW400 genotype can be categorized as high yielding with low protein content for bread making (Orloff et al., 2012)

The observed negative, but a strong correlation (r =-0.91) between grain yield and starch (Figure 5) is contrary to what is widely expected (Aliu et al., 2012). The cause of this negative relationship is not known. However, there is a need for further studies and establish the inherent physiological cause.

Conclusion

The study showed that application of nitrogen on UW400 bread wheat genotype resulted in an increase in grain yield and break flour. The agronomic N rate required ameliorating N stress and obtaining high break flour and grain yield is 60 kg ha^{-1}. There was a direct negative effect of N rate on the level of grain starch in the grains; while no significant effect existed between nutrient rates and grain crude protein levels. As a result, N application caused a crude protein to starch ratio of 0.149-0.192.

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