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Grain Amaranth Yield Response to Nitrogen Fertilization

Matthew Wohlgemuth Blair[1]*, Lucas Mackasmiel[1], Laxmi Prasad Joshi[1], David Hickok[1,2]

[1]Department of Agricultural Science & Engineering, Tennessee State University, Nashville, TN, United States
[2]Department of Plant Breeding, School of Integrated Plant Science, Cornell University, Ithaca, NY, United States
Email: *mblair@tnstate.edu

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Abstract

Grain amaranths are a group of pseudo-cereal species known for drought and heat stress tolerance, high nutritional quality and potential as forage and feed for animals or as a milled or popped seed for baking and industrial processes for human food. Like most row crops, the grain amaranths have a high requirement for soil nitrogen (N) and other types of fertility to grow to their full potential. The goal of this study was to test three levels of urea fertilization (0, 115 and 230 kg/ha N) and four grain amaranth genotypes, two from species *A. cruentus* and the USDA and two from species *A. hypochondriacus* and SSE, for yield components, including seed weight, proportion seed per panicle, plant height, panicle length, panicle width and panicle weight. The genotype USDA107 was observed to grow taller and had longer but thinner panicles compared to USDA127 as well as SSE42 and SSE30 with each of these genotypes being shorter but with wider panicles. These contrasts were stable across fertilization levels. An increase in the yield potential of seed weight and proportion seed per panicle was seen at high dose compared to mid-dose or control, unfertilized treatments. Correlations were positive and highly significant for all traits except panicle length and proportion seed or panicle length and width which were negatively correlated. We expect these novel results to spur further study of grain amaranths in the southeastern United States.

Keywords

Amaranthus cruentus, *A. hypochondriacus*, Nitrogen Fertilization, Panicle Traits, Yield Potential, Urea Fertilizer

1. Introduction

Grain amaranths are made up of the Mexican species *Amaranthus cruentus* L. and

A. hypochondriacus L. as well as the South American *A. caudatus* L., with the first two adapted to lowland environments and the latter found in highland tropics. These dicotyledenous grain crops are all considered orphan crops due of their being under-researched. However, pseudocereals like these grain amaranths can be very important crops for world food security, as they have 15% or more protein, which is higher than corn, and are not from the same plant family as typical cereals, like wheat [1] [2]. The grain amaranths are native to Latin America but have become important in parts of Europe, East Africa and South Asia [3]. They are also of interest because they have high abiotic stress tolerance [4]. They are among the easiest-to-prepare grains, as they can be popped, milled or boiled for direct consumption. In nutritional terms, pseudocereals provide a better balance of amino acids, vitamins and micronutrients than cereals [5]-[7].

In addition, the amaranths are multi-functional crop species. They can be grown as seedlings for sprouts. Their leaves are often consumed as vegetables in many communities and marketable fresh or dry [8]. Leaves, stems and flower heads with seeds can be a successful fodder or feed for either silage or fresh consumption by animals as diverse as pigs and cattle. After harvesting, dry grains can be fed to chickens providing additional food security via eggs and meat [9] [10]. Truly, miracle plants, the amaranths can play a much larger role in many aspects of US and global agriculture [5]. Half a decade ago, a publication called grain amaranths the "zero to hero" crop because of its success around the globe from its humble origins in Mexico [4]. Germplasm diversity for amaranth is high in the original homeland of Latin America [7] [11]-[13].

In terms of general agricultural systems, grain amaranths and quinoa as pseudocereals introduce diversity into crop rotations. Grain amaranth species are naturally resource-efficient plants due to their C4 photosynthetic pathway, rapid growth, and low water requirements [14]. Quinoa and lambs quarter relatives of amaranths have the less-efficient C3 photosynthesis and less heat tolerance, but they are also hardy plants with drought and salt stress tolerance increasingly needed by modern agriculture, allowing efficient and lower-cost irrigation [15]. They can serve as a cover crop just like buckwheat and given their small-seededness do not involve high seed costs found for other species of winter, spring, summer or even fall season crops [16].

The purpose of this study was to evaluate the agronomic performance of four grain amaranth genotypes from two sources (USDA and SSE) and from two species (*A. cruentus* and *A. hypochondriacus*) for response to nitrogen fertilizer provided in the form of urea at a site typical of the climate of the Southern states of the USA. This is one of the first reports of growing grain amaranth under the environmental conditions relevant to the Mid-South region and complement studies of vegetable types and evaluations further north, east and west in the USA. In addition, we treat the grain amaranths as an agronomic, row crop with direct seeding and between-row tillage rather than treating it as a horticultural crop, typical of experiments with it in other countries.

2. Materials and Methods

2.1. Plant Materials

Four cultivars of grain amaranths were used in the experiment, with two from a germplasm collection at Seed Savers Exchange (SSE, Decorah, IA) and two others from the United States Department of Agriculture (USDA) North Central Plant Introduction Center (Ames, IA). These accessions were selected from larger germplasm sets from each gene bank where the best yielding lines were determined under un-fertilized conditions in a previous, single-row grow out by [6] for agro-morphological evaluation at the Tennessee State University (TSU) farm described below. The four genotypes used in this study included two SSE entries: SSE30 (Rodale 124) and SSE42 (Mexican) both of the species *Amaranthus hypochondriacus;* and two GRIN entries: USDA107 (PI-643043, a.k.a. RRC420) and USDA127 (PI-649506, a.k.a. RRC483), both collected by the Rodale Research Center (RRC), both from Mexico, and both identified as *A. cruentus* accessions. These latter lines have been maintained at the USDA gene bank and have their original RRC name and plant introduction (PI) code added in parenthesis.

2.2. Planting Location, Experimental Design and Plot Management

The experiment was conducted at the Agricultural Research and Education Center (AREC) experimental farm for TSU located in Nashville, TN at the geo-coordinates: 36.1758 N-86.8261 W. The soil type was an Armour silt loam (of Inceptisol order), with 2 to 5 percent slope, which is typical of floodplain soils in the Cumberland River Valley. A portion of the field at the top of the slope, which was known for lower fertility was selected for this variety trial/fertilizer experiment. The experiment was laid out in a split-plot with randomized complete block design (RCBD) with four replications, where varieties were assigned to main plots and fertilizer levels to subplots. The planting date was May 15th, 2020 at two weeks after last freeze for our region. Direct seed planting was at a rate of 2.5 g per 4 meters (m) of row length. Three rows planted with this length represented a plot and a double row alley was left between plots to avoid cross contamination of seed or movement of fertilizer. Soil preparation was done with two rototilling passes after a single plowing pass using a John Deere 1023e sub-compact tractor outfitted with a RC13 series Frontier Rotary Tiller. Row formation was with a four-tine tiller with 30 cm spacing that allowed us to create straight rows along the gradient after soil preparation was complete and to mark where we were planting the small seed of grain amaranth. Total plot size was 3.6 m^2 with three rows per plot separated by 30 cm between rows, subplots separated by 60 cm in alleys and 100 cm between plots at their row ends. Planting was done into the opened rows the day after a rainfall event to stimulate germination. Gentle raking was used to cover the seed after planting. A short watering-in of the seed into the soil with a hose attached to a city water hydrant was used for initial irrigation (5 cm of water applied) at one week after planting. During the experiment plots were hand weeded for any off type or weedy amaranth or any grass seedlings and hoeing was used to maintain inter-row spaces free from weeds as well.

2.3. Fertilizer Levels

Fertilization was applied at three nitrogen (N) treatment levels based on urea fertilizer equivalent of full dose (230 kg/ha. a.k.a. 2X), half dose (115 kg/ha of N, 1X) and control (0 kg/ha, 0X). The source of urea was 46-0-0 NPK fertilizer and the three respective treatments corresponded to 500, 250 and 0 kg/ha of urea. Urea was used instead of a mixed fertilizer, because previous soil analyses had found the native soils in AREC experimental station farm to be sufficient in phosphorus (P) and potassium (K), slightly acidic to near neutral (pH = 5.7) and moderate in carbon but low in nitrogen [17]. Fertilizer applications were done as side-dressing to plants after the first weeding, approximately three weeks after planting, with the urea in the side row gently covered by hand-hoeing. The experiment followed a fallow growth period from the previous year. In that previous year, the section had been mowed to maintain lower growth plants and to avoid pigweed and other amaranth plants that can be weedy in our region. The only dicotyledonous weeds other than pigweed to contend with were lambsquarters seedlings. The rotation was used to deplete any of nitrogen in the soil. No legumes were observed in the plot prior to planting amaranth.

2.4. Harvesting and Data Analysis

Plants in the grain amaranth plots were harvested starting in late September in the order in which they matured. Subsampling consisted of 3 plants per plot, which were randomly chosen from the three rows of the plot. Panicles of the individual plants were evaluated and then cut-off so that they could be hand-threshed and winnowed through a sieve and air cleaner to get clean seed. This was done with each variety and fertilizer level, and the plants were measured for six traits: panicle length (RL), panicle width (PWd) and panicle weight (PWt), plant height (PH), seed weight (SW) yield and proportion seed within panicle (PS). This last trait was calculated with the formula: (SW/PW) to obtain a proportional value. Data were organized in MS Excel (Microsoft Corp., v. 2022). Analysis of variance (ANOVA) was conducted assuming fixed genotypes, fixed fertilizer levels and random replicates using R version 4.5.1 (R Core Team, 2025). Averages ± standard deviations (SD) were graphed with R software for each significant ANOVA result and different letters above the bar plots represented significant differences from a Tukey's test.

3. Results

3.1. ANOVA Results

ANOVA results show significant genotype effects for plant height (PH, $P < 0.05$) and the two parameters of panicle dimension (PL, $P < 0.001$ and PWd, $P < 0.05$). The varieties differed significantly in proportion seed in the panicle (PS, P <

0.001). However, yield as measured by panicle weight (PWt) or seed weight per plant (SW) did not show significant genotypic differences (Table 1), showing that

Table 1. Analysis of variance for traits measured in varietal and fertilizer level trial of four grain amaranth cultivars grown at the Tennessee State University experimental farm in Nashville, TN.

Effect	Sum Sq	Mean Sq	Num DF	F value	*P*-Value[a]
***Seed Weight* (*SW*)**					
Genotype	821.3253	273.7751	3	1.5671012	
Fertilizer	1,810.5030	905.2515	2	5.1817008	**
Genotype × Fertilizer	816.6851	136.1142	6	0.7791238	
Residual	22536.508	174.701	129		
***Panicle Weight* (*PWt*)**					
Genotype	3,461.332	1,153.777	3	1.106169	
Fertilizer	19,664.242	9,832.121	2	9.426422	***
Genotype × Fertilizer	6,271.821	1,045.304	6	1.002172	
Residual	132476.031	1043.118	127		
***Panicle Length* (*PL*)**					
Genotype	2,655.2074	885.06913	3	12.317201	***
Fertilizer	459.2326	229.61632	2	3.195491	*
Genotype × Fertilizer	445.8463	74.30771	6	1.034115	
Residual	9269.469	71.856	129		
***Panicle Width* (*PWd*)**					
Genotype	810.4691	270.1564	3	3.717370	*
Fertilizer	463.9050	231.9525	2	3.191682	*
Genotype × Fertilizer	1,188.9661	198.1610	6	2.726709	*
Residual	9374.952	72.674	129		
***Plant Height* (*PHt*)**					
Genotype	4,785.294	1,595.0981	3	3.404440	*
Fertilizer	2,654.988	1,327.4942	2	2.833289	°
Genotype × Fertilizer	3,701.008	616.8347	6	1.316519	
Residual	60440.982	468.534	129		
***Proportion Seed* (*PS*)**					
Genotype	0.080045	0.026682	3	6.440554	***
Fertilizer	0.001428	0.000714	2	0.17234	°
Genotype × Fertilizer	0.162805	0.054268	6	13.09966	°
Residual	0.526	0.004	129		

a. *P*-values: °, *, **, *** represent detected probability significance levels of 0.1, 0.05, 0.01 and 0.001, respectively for the respective traits genotype, fertilizer or genotype x fertilizer effects.

although the varieties had differences in panicle size and ratio of seed in the panicle as compared to chaff they did not differ in underlying productivity. On the other hand, fertilizer effects were highly significant for the yield traits of PWt ($P < 0.001$) and SW ($P < 0.01$), for panicle length ($P < 0.05$) and width ($P < 0.05$) as well as nearly significant for PH and PS ($P < 0.1$). Finally, genotype x fertilizer interactions were only significant ($P < 0.05$) for width of panicle (PWd) versus close to significant ($P < 0.1$) for proportion seed (PS), but non-significant for the remaining traits including PH, PL, PWt, PS, and SW. Overall, this suggested stable cultivar and fertilizer effects without interaction of the treatments of genotype and fertilization level for the majority of traits.

3.2. Plant Height, Yield and Panicle Differences between Varieties

Plant height was significantly different between varieties across the three fertilizer levels with USDA 107 being the tallest and SSE42 being the shortest, and the other two genotypes (SSE30 and USDA127) being intermediate (Figure 1). This height difference was reflected in similar ranking for the four genotypes for panicle length (PL). While USDA107 had the longest panicles, it was also the thinnest in profile with the lowest panicle width (PW). SSE30 was characterized by widest panicles with SSE42 and USDA127 intermediate.

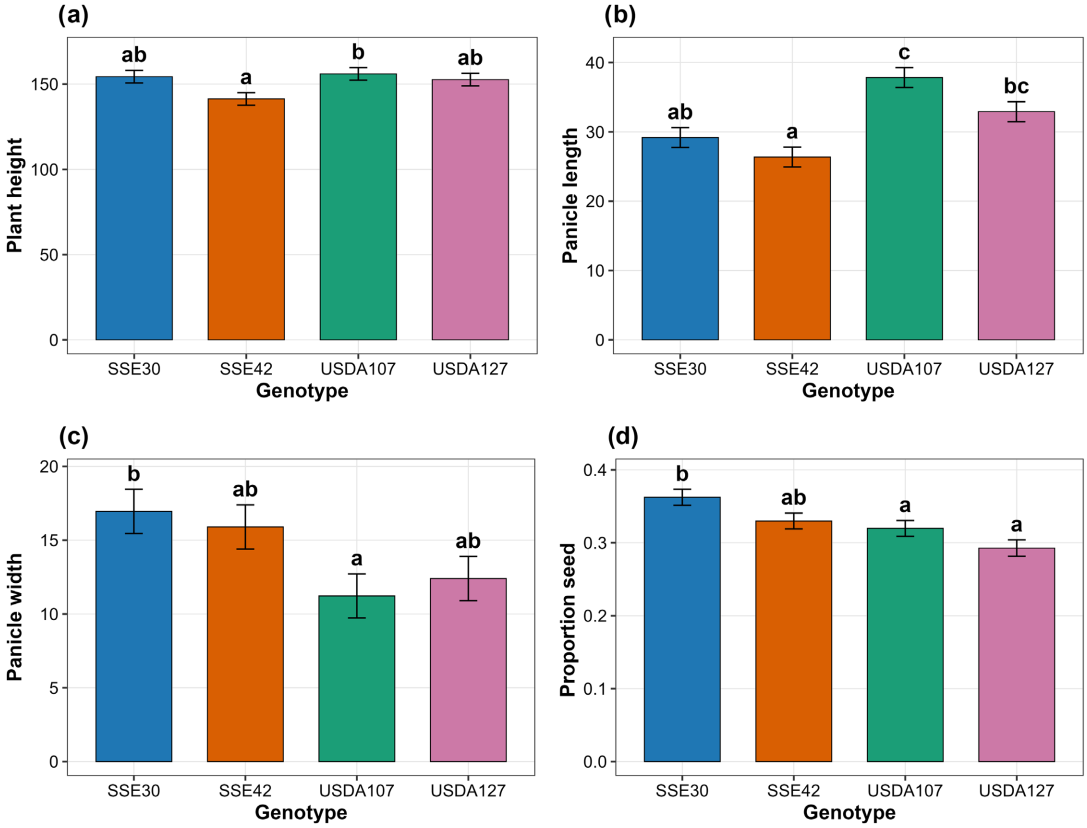

Figure 1. Genotype values for four grain amaranth plant traits measured across three fertilization treatments where varietal differences were significant: (a) plant height (PH); (b) panicle length (PL); (c) panicle width (PWd); and (d) proportion seed in the panicle (PS).

Within fertilizer levels the differences were born out as well (**Table 2**), with USDA107 having the longest panicle length (PL) at all three levels of fertilization (control 0, 250 and 500 kg/ha urea). Panicle width (PWd) was inversely the thinnest for USDA107 at 500kg/ha urea but second thinnest to USDA127 at 0 and 250 kg/ha urea. The two SSE entries were wider for panicles, which could be a species level difference compared to the USDA entries.

Table 2. Genotype values for six plant traits measured across three fertilization treatments for four grain amaranth cultivars grown at Tennessee State University experimental farm in Nashville, TN.

Fertilizer Level kg/ha N	*Trait* (*measure*)/Germplasm Cultivar					*Trait* (*measure*)/Germplasm Cultivar				
	Seed Weight (*SW*) (*g*/*plant*)					*Proportion Seed* (*PS*) (0 - 1)				
	SSE 30	SSE 42	USDA 107	USDA 127	Average	SSE 30	SSE 42	USDA 107	USDA 127	Average
0 kg/ha	22.53^{a}	12.7^{a}	13.61^{a}	10.38^{a}	14.81	0.36^{ab}	0.33^{ab}	0.30^{ab}	0.30^{ab}	0.32
115 kg/ha	18.78^{a}	13.56^{a}	22.30^{a}	15.87^{a}	17.63	0.35^{ab}	0.32^{ab}	0.36^{ab}	0.28^{a}	0.33
230 kg/ha	25.41^{a}	22.29^{a}	21.63^{a}	23.99^{a}	23.33	0.38^{b}	0.34^{ab}	0.30^{ab}	0.30^{ab}	0.33
Average	22.24	16.18	19.18	16.75	18.59	0.36	0.33	0.32	0.29	0.33
	Panicle Length (*PL*) *cm*					*Panicle Width* (*PWd*) *cm*				
0 kg/ha	27.59^{ab}	25.49^{ab}	33.92^{abc}	32.63^{abc}	29.91	20.98^{c}	14.64^{abc}	8.79^{ab}	7.21^{a}	12.91
115 kg/ha	27.00^{ab}	24.54^{a}	37.04^{bc}	34.50^{abc}	30.27	14.82^{abc}	14.39^{abc}	12.08^{abc}	9.88^{abc}	12.79
230 kg/ha	32.96^{abc}	29.08^{ab}	42.54^{c}	31.63^{abc}	34.05	15.06^{abc}	18.65^{abc}	12.79^{abc}	20.12^{bc}	16.66
Average	29.18	26.37	37.83	32.92	31.41	16.95	15.89	11.22	12.4	14.79
	Panicle Weight (*PWt*) *g*					*Plant Height* (*PH*) *cm*				
0 kg/ha	58.31^{ab}	36.74^{ab}	46.33^{ab}	33.09^{a}	43.62	159.30^{a}	135.37^{a}	154.02^{a}	140.69^{a}	147.85
115 kg/ha	49.45^{ab}	41.00^{ab}	59.37^{ab}	53.60^{ab}	50.86	153.27^{a}	137.66^{a}	151.31^{a}	152.90^{a}	148.78
230 kg/ha	63.99^{ab}	63.87^{ab}	75.17^{ab}	82.92^{b}	71.13	150.51^{a}	150.88^{a}	162.66^{a}	164.30^{a}	157.59
Average	57.25	47.2	60.29	56.72	55.20	154.36	141.3	155.99	152.63	151.41

1. Letters indicate Tukey's test differentiation between fertilizer and genotype treatments. All values represent the means of three plants subsampled per plot.

In terms of PWt, the two USDA genotypes responded better to fertilization being higher than the two SSE genotypes at 250 kg/ha and 500 kg/ha urea but lower at control fertilization of 0 kg/ha urea. Seed weight per plant (SW) difference was not significant in the genotype x fertilizer level interaction but were for proportion seed per panicle (PS).

3.2. Differences between Fertilizer Levels

The effect of fertilizer level was significant for three of the six traits. The 500kg/ha level of urea fertilization resulted in significantly higher PWt and SW compared to the 250 kg/ha and 0 kg/ha levels, respectively (**Figure 2**). PWt averages were 71.2, 50.9 and 43.6 g and SW averages were 23.3, 17.7 and 14.8 g for the three fertilizer levels from highest to lowest/control. This reflected the longer panicle length where the PL trait showed a significant difference of 35 cm compared to 30 cm, between 500 kg/ha versus 0 kg/ha fertilizer levels, respectively. The other panicle trait of width (PWd) did not show significant differences for fertilizer level with Tukey's test despite the significance at $P < 0.05$ level in the ANOVA results for this treatment. Proportion seed per panicle (PS) differences between genotypes were very similar to those for PWt and SW with all three highest in SSE30 at 230 kg/ha N (or 500 kg/ha urea). However, USDA107 was also high at 115 kg/ha N (250 kg/ha urea).

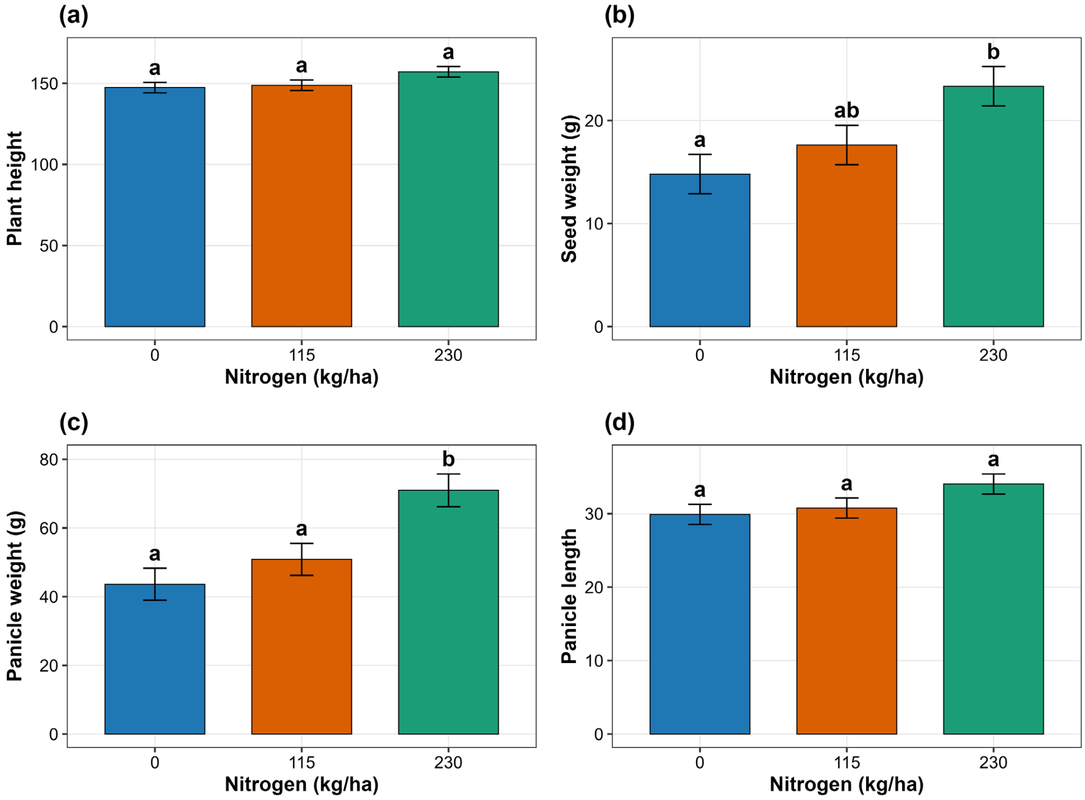

Figure 2. Fertilizer effect on yield and panicle traits for four grain amaranth cultivars evaluated at the TSU experiment station in Nashville, TN. Signficance differences from Tukey's test indicated by letters above bars. Probability levels for traits' ANOVAs treatment effects are as follows: PWt (P < 0.001), SW (P < 0.01), PL (P < 0.05) and PWd (P < 0.05).

3.3. Correlations between Traits

Overall plant height (PH) was correlated positively with all other traits (**Figure 3**) ranging from r = 0.69 ($P < 0.000$) for PWt to r = 0.24 ($P < 0.003$) for PWd. Seed weight (SW) was also correlated significantly and positively with PH (r = 0.63, $P < 0.000$). The PH and PL traits were positively correlated at a lower level (r = 0.44, $P < 0.000$) as were PH and PS (r = 0.28, $P < 0.001$). Panicle traits were significantly correlated amongst each other. As examples, PWt and PL (r = 0.40), and PWt and PWd (r = 0.44) were highly significant ($P < 0.000$), positively correlated. Interestingly panicle length and width were negatively correlated (r = −0.48, $P < 0.000$), indicating some sort of compensation mechanism between these traits which in turn influence overall seed yield measured as SW (r = 0.34 and r = 0.45) more than as PS (r = 0.03 and r = 0.23), the former of these two correlations not significant. Finally, SW and PS were positively and significantly correlated (r = 0.53, $P <$ 0.000).

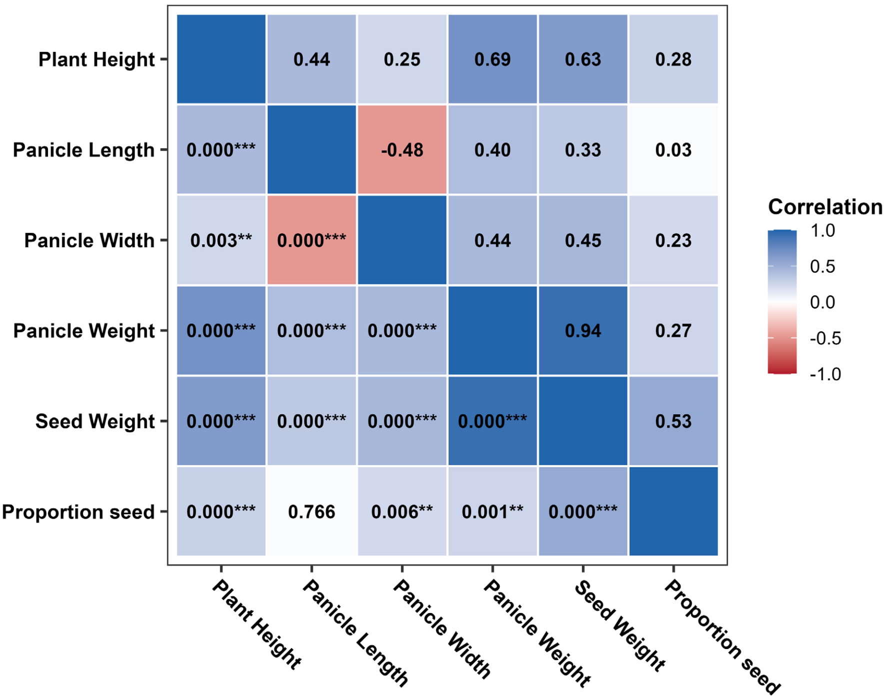

Figure 3. Correlation values (above diagonal) and probability values (below diagonal) for panicle and yield traits collected on four grain amaranth cultivars across three fertilization levels upon evaluation at the TSU experiment station in Nashville, TN. Scale to the right indicates level and significance of the correlation and whether it is negative (orange to red) or positive (light to navy blue).

4. Discussion

Only a few fertilizer trials have been done for grain amaranth in North America or for that matter around the world as it is typically grown as a rustic crop without modern inputs. In the USA, a landmark study [18] used cultivars 'Plainsman', D136 and K266 to compare 0, 45, 90, 135 and 180 kg/ha of N fertilization, finding that the intermediate levels were the best with higher fertilization causing excess plant height and delayed crop maturity on a site dependent basis. Since in Tennessee, we are located at a lower latitude and therefore have a long season that can accommodate any maturity delays, we decided to use 115 and 230 kg/ha N applied as urea to observe differences in yield characteristics. We also decided to take data on panicle characteristics on a per plant basis to avoid edge effects that can lead to taller plants and yield difference between varieties when different size plants have a larger area to grow and to scavenge fertility from. While Myers [18] used breeding lines from complex crosses we used pure *A. hypochondriacus* accessions from SSE and pure *A. cruentus* accessions from USDA. With these differences, we found that the 230 kg/ha N fertilization level produced more in terms of panicle weight or seed weight per plant than the half dose or control level of nitrogen. This is consistent with studies in Arkansas [19] and Minnesota [20] where yield responses were observed with up to 200 and 267 kg/ha of N, respectively.

Our selection of the SSE and USDA entries was part of a local breeding program for high-yielding, full-stature grain amaranths. Some of the available lines in the USA include shorter, semi-dwarf genotypes such as Plainsman which was selected in Nebraska to grow less than two feet tall and to allow for mechanical harvest. However, given the high rainfall in Tennessee we preferred to have taller plants that would avoid soil contact, but this has meant that we need to harvest panicles from the tops of plants [12]. We also preferred light colored panicles that would be easy to evaluate for clean seed. Apart from these entries, grain amaranth germplasm is extensive and our program has evaluated both a core collection of 260 entries held in the United States Germplasm Repository (GRIN) and over 30 landraces from the non-governmental seed advocacy program of SSE [7]. The genotypes have been evaluated both phenotypically and genotypically to find elite, adapted lines that can be useful in the Southern states [11].

In Latin America, where the *A. cruentus*, *A. caudatus* and *A. hypochondriacus* grain amaranths originate from and where they grow on tropical highlands or sub-tropical lowland soils, fertilization usually increases yields [21]. This is to be expected as these soils usually are deficient in nitrogen and other essential nutrients. In some other locations, such as in Guatemala, the crop is grown on high fertility, volcanic soils and the responses to fertilization are less notable [22]. In Asia, the grain amaranths, are secondary to vegetable types; except in Nepal where the former are important and found to be responsive to N-P-K fertilization [23].

In Africa, some vegetable entries of *A. hypochondriacus* are more extensively studied for response to N fertilization than Asian species or other grain species [24]. Dual purpose vegetable and grain amaranth varieties are popular in East Africa, requiring careful analysis in that region for the cultivars that have been studied for fertilizer needs [25]. Meanwhile in West Africa, fertilization studies refer to vegetable amaranths which are cut for leafy shoots multiple times a season, using up fertility of the soil very quickly [24] [26]. For example, the vegetable yield of a local amaranth cultivar was studied in Nigeria using three levels of farmyard manure as an organic fertilizer source and inorganic compound 20-10-10 NPK applied at 0, 150, 300 and 450 kg/ha with strong linear regression between application rate and yield [27]. Grain amaranth in Kenya responded best to 87.5 kg/ha N from inorganic fertilizer supplemented with manure at 9 t/ha [28]. In a European study, application of 50 and 100 kg/ha N to one *A. cruentus* and one *A. hypochondriacus* cultivar in Croatia led to significant increase of seed yield compared to control (0 kg/ha N) in a dry year but not in two wet years [29]. Therefore, year x year environmental conditions affect the yield response to N.

In summary for this study, we found fertilization with moderate to high doses of urea to be useful for increasing the yield capacity of grain amaranth genotypes in our location and under our weather conditions of a lengthy, hot summer season. We found consistency across four varieties, two each representing the main heat-adapted species of *A. cruentus* and *A. hypochondriacus*. Further testing is recommended with more genotypes or more testing sites and years. In terms of agronomic utilization, producers from a large region across the Southeastern USA, from mid-Atlantic states of Maryland or Delaware, through Virginia, all the way to Oklahoma and Texas could benefit from research like ours on grain amaranths, as a crop with natural heat tolerance and low water requirements [5]. One previous test of two *A. cruentus* grain amaranths and four vegetable amaranths found significant increase in leaf nitrates and leaf crude protein and decrease in leaf neutral detergent fiber in response to N fertilization at rates of 50 and 100 kg/ha N which could affect forage use of amaranth leaves by ruminants [30]. Amaranth grain or leaves can also be used to extract squalene, a valuable oil used in skin products [31]. Furthermore, grain amaranth seed has potential for feeding chickens at up to 20% or 40% feed [9] [10] [32]. The potential of grain amaranths for food, feed, industrial or forage use, remains underutilized in southern USA due to limited regional data on the agronomic performance and grain quality traits for genotypes and soils available here. Alternative sources of nitrogen for organic production of amaranths should also be studied and could include farmyard manure, legume precursors, or rotation onto fallowed ground [33]. Any of these fertility trials with the levels of nitrogen identified in this study across various states with these or similar genotypes to ours would be useful for obtaining data on grain quality, forage potential and agronomic performance of grain amaranths under conventional or organic methods.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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