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Full Length Article

Adaptability and Yield Potential of Different Species of Amaranth under Semiarid Conditions

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Abstract

Amaranth, being a nutrient-rich and climate resilient crop, can be a solution to improve nutritional quality and food security for increasing population. Aims of this study were to check the adaptability and yield potential of amaranth under semiarid climate conditions of Pakistan. This two-year field experiment was conducted at Directorate Research Area, University of Agriculture, Faisalabad. Germplasm of amaranth (ten genotypes) was imported from USDA and grown under semiarid environment to compare their phenology, leaf biochemical analysis and yield attributes in order to access its adaptability. Significant variations were observed among the genotypes for yield related attributes, leaf chlorophyll contents and phenology. Among genotypes, maximum grain yield was produced by PI 642733 followed by PI 619265, PI 636194 and Ames 15204. This was linked with stay green character (more leaf chlorophyll contents) of genotypes for longer period, as depicted by more seed setting periods of high yielder genotypes. Genotypes completed seed setting between 112 to 128 days after emergence. Furthermore, seed protein contents ranged between 11.73 to 19%. Genotypes PI 642733, PI 619265, PI 636194 and Ames 15204 were found promising and recommended to be grown in *Rabi* crop season in Faisalabad conditions. Huge diversity observed in the germplasm of amaranth which opened new avenues for the selection and production of suitable germplasm under different agro-ecological zones of Pakistan. © 2020 Friends Science Publishers

Keywords: Amaranth; Future smart food; Genotypes; Phenology; Seed yield

Introduction

Impacts of climatic conditions result changing in global temperature, low rainfall, loss of biodiversity, multiple biotic and abiotic stresses on livestock, crops and land degradation (Reddy 2015). By 2050, it is estimated that people are now moving towards South Asia and Sub-Saharan Africa that makes these regions more saturated by addition of 2.4 billion of people. Agriculture is the main economic sector for these regions and also provides employment to the people of these regions. A study reveals that on an average about 20% of population is food insecure (Lipper *et al.* 2014). At present, new plants are being introduced by breeders and agronomists that have more adoptability potential to grow in all types of terrestrial environment on the earth. So, it is need of hour to introduce naturally stress resilient plants (NSRPs) that can survive under all type of stress conditions and unfavorable environment that are unfriendly for most of the crops.

The NSRPs are sometimes known as extremophiles plant like pearl millet (*Pennisetum glaucum*), quinoa (*Chenopodium quinoa*) and grain amaranth (*Amaranthus hypochondriacus*) (Zhang *et al.* 2018) because these plants can survive under harsh stressful environmental conditions. These crops are also known as superfood crops, so they are helpful to minimize the pressure of food insecurity (Massawe *et al.* 2016). In most areas of the world, amaranth is being increasingly recognized that has a more potential to play an important role as alternative food grain. Because of its more degree of phenotypic plasticity, nutritional profile,

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high adaptability in various environmental conditions and ability to improve food security, it can be grown in marginal lands (Achigan-Dake *et al.* 2014; Alemayehu *et al.* 2014). In maximum areas of Asia and Africa, amaranth is consumed as a food crop in the form of both leafy vegetables and grains (Brenner *et al.* 2000). There are more than 87 species of amaranth are globally exist and three species are area characterized as a grain and about 17 species as an edible leaf form (Grabben and Denton 2004). *Amaranthus cruentus* and *Amaranthus hypochondriacus* of these grain species are originated from North and Central America and remaining one grain specie that is *Amaranthus caudatus* is originated from South America (Rosa *et al.* 2009). Main proteins in amaranth are albumin and globulin and their concentration varies from 13–19% (Gorinstein *et al.* 2002).

On the basis of nutritional value some essential bioactive compounds, flavonoids, tannins, anthocyanins, phytosterols and phenolic acids are also present in grains of amaranth (Ryan *et al.* 2007). In amaranth, bioactive compounds play an important role as a preventive agent in different chronic diseases like diabetes, urinary problems, cancer, cardiovascular problems and hypercholesterolemia (Abugoch 2009). Amaranth grains are more effective in hypo-cholesterolemic index and have more antioxidant value (Gorinstein *et al.* 2002). About 5 to 13% of lipids are present in it and a high amount of unsaturated fatty acid (linoleic acid) is also present (Schoenlechner *et al.* 2008; Alvarez-Jubete *et al.* 2010). In amaranth, maximum amount of vitamins and minerals are present like sodium (Na), calcium (Ca), magnesium (Mg), sulphur (S), phosphrous (P), potassium (K), manganese (Mn) and iron (Fe) and Vitamin B complex (Alvarez-Jubete *et al.* 2010).

Amaranth is a C4 plant, a pseudo-cereal crop and can raised under warm and bright light environmental conditions. Temperature needed for amaranth growth is varies from 25–30°C (Schippers 2000). Amaranth species are able to survive under high drought and heat because of C_4 photosynthetic machinery due to lack of photorespiration damages and better CO_2 reserves Amaranth plant shows good recovery under water-deficient soil by conserving water through minimizing the transpiration water losses and lack of photorespiration (Louh *et al.* 2014). In the study of Omami *et al.* (2006) and Costa *et al.* (2008) it is clear that mild to moderate salinity does not adversely affect the yield of amaranth plant. Amaranth is considered as a high valuable crop because of its climate proof nature and good quality of nutritional profile.

Amaranth can replace the wheat (*Triticum aestivum* L.) crop in areas from which it has already disappeared due to the abiotic stresses and help to improve the quality of food intake. Due to wider adaptability in diverse soil and climatic conditions and unique nutritional profile, exotic germplasm of this crop was tested to check adaptability and yield potential in agro-climatic conditions of Faisalabad- Pakistan.

Materials and Methods

Plant material and design

The seeds of ten genotypes were collected from United States Department of Agriculture (USDA). Ten genotypes were assessed for their adaptability and yield potential under agro-climatic conditions of Faisalabad. Origin and complete detail of genotypes are given in Table 1. The experimental design was randomized complete block design having three replications.

Experimental details

This two years field trial (2017-18 and 2018-19) was executed at Directorate of Farms in University of Agriculture, Faisalabad Pakistan (73.87°E, 31.87°N; altitude 184.3 m.a.s.l.). The climate of Faisalabad is semi-arid and sub-tropical. Under experiment, all of the weather data regarding rainfall, temperature and relative humidity are presented in Fig. 1. Chemical and physical characteristics of the soil are presented in Table 2 and analysed according to the standard protocols described by the Estefan *et al.* (2013).

For conserving soil moisture and obtaining maximum emergence two ploughing at a depth of 12 cm was done followed by one planking. Amaranth crop was sown on the tip of each ridge spaced apart by 22.5 cm with hand dibbled method on 21 November 2017 and 25 November 2018. Seeds were sown at a depth of 2-3 cm using seeding rate of 7.5 kg ha^{-1}. Each experimental unit was consisted of four ridges with each ridge length of 5 m and ridge to ridge distance was 75 cm. The two outer ridges were acting as guard rows, and the terminal amaranth plant was placed at the beginning and end of central rows also act as guard plants. Urea (46% N), diammonium phosphate (18% N and 46% P_2O_5), and sulphate of potash (50% K_2SO_4) were used as fertilizers and applied at the rate of 75:50:50 NPK kg ha^{-1}, respectively. During the land preparation the half dose of N and full dose of P and K were added. At the time of flowering phase, remaining dose of N was applied. Two weed hoeing were done manually 30 and 60 days after sowing from experimental field. Yield was calculated from a total plot size of two ridges by 4.75 m in length. Harvesting was done on 16 April 2018 and 18 April 2019.

Phenological traits

On the basis of daily field visits, all phenological traits such as, total crop duration, bud initiation, milking stage and seed setting were observed.

Leaf Chlorophyll and carotenoid contents analysis

For chlorophyll determination procedure of Nagata and Yamashta (1992) was followed, while carotenoids were measured using the standard protocol of Davies (1976).

Table 1: Genotypes, passport data and phenotypic characteristics of amaranth species used in the experimentation

Genotypes #	Species	Origin
PI 636194	*Amaranthus hypochondriacus*	Mexico, Federal District
Ames 1970	*Amaranthus cruentus*	Ghana
PI 642733	*Amaranthus hypochondriacus*	Nepal
PI 649305	*Amaranthus hypochondriacus*	China, Heilongjiang
PI 481272	*Amaranthus hypochondriacus*	India
PI 619265	*Amaranthus hypochondriacus*	Nepal
Ames 2031	*Amaranthus cruentus*	China
Ames 18034	*Amaranthus hypochondriacus*	Nepal
Ames 15204	*Amaranthus hypochondriacus*	India
PI 654390	*Amaranthus caudatus*	United States, Lowa

Table 2: Physical and chemical analysis of soil of field trial site

Soil characteristics	Unit	2017–2018		2018–2019	
Depths	Cm	0–15	16–30	0–15	16–30
Texture	Class	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Organic matter	g kg^{-1}	0.081	0.067	0.073	0.062
pHe	-	8.0	8.1	8.2	8.0
Electrical conductivity (ECe)	dS m^{-1}	0.72	0.53	0.69	0.52
Total nitrogen	g kg^{-1}	0.0041	0.0035	0.0038	0.0034
Available phosphorus	mg kg^{-1}	7.5	5.5	7.1	5.2
Extractable potassium	mg kg^{-1}	135.5	121.1	137.5	122.7

At the time of panicle emergence stage, fully expanded young leaves were harvested from each experimental unit. A 0.5 g leaf sample was grounded in 10 mL of 80% acetone using pestle and mortar, grounded material was put in falcon tube and centrifuged for 15 minutes at 4000 rpm, resulted supernatant was subjected to take absorbance at 663, 645 and 480 nm using spectrophotometer (UV 4000). Later on, chlorophyll a, b, total chlorophyll and carotenoids were calculated using following formulae:

$$Chlorophyll\ \mathrm{a}\ (mg/g) = \frac{[(0.0127 \times A663 - 0.0029 \times A645) \times 100]}{0.5}$$

$$Chlorophyll\ \mathrm{b}\ (mg/g) = \frac{[(0.0229 \times A645 - 0.00468 \times A663) \times 100]}{0.5}$$

$$Carotenoid\ (mg/g) = \frac{[A480 + 0.114\ (A663) - 0.638\ (A645)]}{2500\ \times 1000}$$

Seed protein contents

Nitrogen content in flour samples was estimated by Kjeldahl's method. Nitrogen percentage was calculated using Equation:

$$Nitrogen\ (\%) = \frac{[\text{Titer of 0.1 NH2SO4} \times 0.0014 \times 250]}{\text{Weight of sample} \times \text{Volume of aliquot sample}}$$

Seed protein percentage was calculated by multiplying percent nitrogen with 5.7.

Yield and related traits

At harvest, ten plants were tagged in each plot to record the yield related attributes such as plant height, number of panicles per plant, terminal panicle length, terminal panicle dry weight and terminal panicle grain yield. After harvesting in each experimental unit, crop plants were tied into bundles and sun-dried in the field for 5 days within respective experimental unit. Plant biomass was recorded by using a spring balance, and by manual threshing grain yield was obtained and recorded with an electric balance. Harvest index (HI) was recorded as the ratio of grain yield to biological yield, expressed as a percentage. From grain yield of each experimental unit, grain sample was taken, and 1000 grain was counted by seed counter and the 1000-grain weight was determined with an electric balance.

Statistical analysis

Data of all traits recorded for two years were pooled and used in further statistical analysis. All recorded data for each parameter were subjected to the SAS 9.1 software to work out an analysis of variance (ANOVA). Data regarding phenological, leaf chlorophyll analysis and yield related attributes are presented in tables with critical values to compare genotypes using Tuckey HSD test at the 5% level of probability. Correlation (Pearson) among studied traits was also calculated using statistical package *Statistix 8.1*.

Results

Yield related attributes

Significant variations ($P \leq 0.001$) were observed among the genotypes for yield related attributes such as plant height, number of panicles per plant and biomass (Tables 3, 4). Among the genotypes, maximum plant height was recorded in ‘Ames 18034’ followed by ‘PI 642733’, ‘Ames 1970’ and ‘PI 481272’, while maximum number of panicles per plant were recorded in Ames 15204 followed by Ames 1970, PI 642733 and Ames 2031 (Table 3). Genotype ‘PI

Table 3: Mean values for yield related attributes in 10 genotypes of amaranth over two years

Genotypes	Plant height (cm)	Number of panicles per plant	Terminal panicle length (cm)	Terminal panicle dry weight (g)	Terminal panicle grain yield (g)
PI 636194	74.75 DE	13.33C	18.02 B	32.07 C	16.50 A
Ames 1970	97.45 BC	19.33 AB	17.32 B	18.22 D	9.53 C
PI 642733	100.38 B	16.67 BC	16.43 B	61.02 A	13.68 B
PI 649305	50.40 F	14.00 C	17.33 B	12.67 EF	4.27 D
PI 481272	96.58 BC	17.83 A-C	17.27 B	14.70 E	4.67 D
PI 619265	69.60 E	14.50 C	16.82 B	42.28 B	11.47 C
Ames 2031	62.33 EF	16.17 BC	24.92 A	11.33 FG	3.20 D
Ames 18034	119.82 A	14.67 C	18.70 B	32.90 C	9.92 2C
Ames 15204	66. 12 E	21.83 A	15.27 B	33.28 C	15.75 AB
PI 654390	86.02 CD	16.83 BC	17.88 B	9.29 G	3.62 D
HSD (0.05)	12.84	4.63	4.26	2.66	2.17

Different letters within the same column indicate statistically significant differences at $P \leq 0.05$

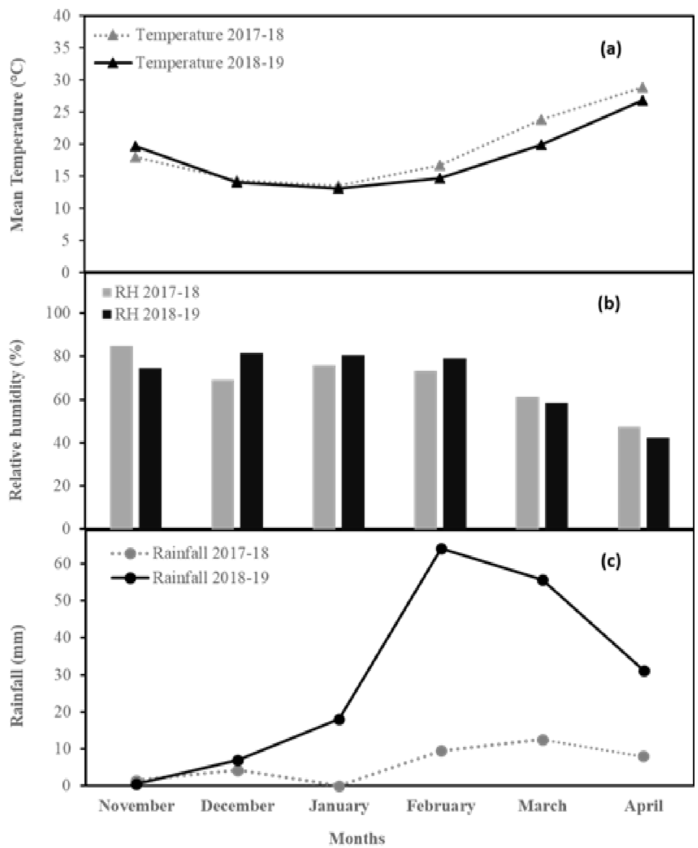

Fig. 1: Temperature, Relative humidity and rainfall during experimental period (Nov–April 2018 and 2019)

619265' produced maximum biomass followed by 'PI 481272', 'PI 636194' and 'Ames 1970' (Table 4).

Moreover, significant variations ($P \leq 0.001$) were recorded among the genotypes for terminal panicle length, terminal panicle dry weight and thousand grain weight and harvest index (Tables 3 and 4). Maximum terminal panicle length was recorded in 'Ames 2031' and remaining all genotypes had statistically similar terminal panicle lengths (Table 3). Maximum terminal panicle dry weights was produced by 'PI 642733' followed by 'PI 619265', 'Ames 18034' and 'PI 636194' while minimum value was noted in 'PI 654390' (Table 3). Furthermore, maximum value of 1000-grain weight was found in 'Ames 18034' followed by 'PI 654390' and 'PI 642733' that was statistically similar and minimum value for this trait was observed in 'PI 636194' (Table 4). Maximum harvest index was noted in 'PI 649305' followed by 'Ames 15204' (Table 4). While among the genotypes, maximum grain yield was produced by 'PI 642733' followed by 'PI 619265', 'PI 636194' and 'Ames 15204' (Table 4).

Panicle and seed colour

Genetic diversity was observed between the genotypes for

Table 4: Mean values for yield related attributes in 10 genotypes of amaranth over two years

Genotypes	1000-grain Weight (g)	Plant biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Harvest index (%)
PI 636194	0.34 E	12005 A	1176 AB	9.80 EF
Ames 1970	0.42 D	11806 A	952 D	8.08 F
PI 642733	0.62 A	7610 CD	1280 A	16.93 B
PI 649305	0.42 D	3556 E	942 D	26.82 A
PI 481272	0.52 C	10056 AB	1265 A	12.78 C-E
PI 619265	0.51 C	8144 B-D	1130 BC	13.90 B-D
Ames 2031	0.50 C	7100 CD	962 D	13.57 B-E
Ames 18034	0.63 A	8788 BC	1000 CD	12.12 DE
Ames 15204	0.55 B	6740 CD	1068 B-D	16.35 BC
PI 654390	0.60 A	6616 D	1066 B-D	16.22 BC
HSD (0.05)	0.03	2085.5	131	3.87

Different letters within the same column indicate statistically significant differences at $P \leq 0.05$

Table 5: Mean values for phenological stages in 10 genotypes of amaranth over two years

Genotypes	Days taken to emergence (days)	Days taken to bud initiation (days)	Days taken to milking stage (days)	Days taken to seed setting (days)	Panicle colour	Seed colour
PI 636194	9.5 BC	44D E	106.5 C	128.5 C	Yellow	Yellow
Ames 1970	8.5 D	46.5 B	100.5 H	115.5 E	Dark pink	Black
PI 642733	10 B	45.5 C	114 A	130.5 A	Green	Black
PI 649305	7.5 E	43.5 E	101 GH	115 E	Yellow	Creamy yellow
PI 481272	9 CD	46.5 B	109.5 B	129.5 B	Yellow	Creamy yellow
PI 619265	9.5 BC	44.5 D	105 D	124.5 D	Purple	Black
Ames 2031	9.5 BC	46.5 B	104 E	111.5 H	Brown	Creamy yellow
Ames 18034	9 CD	48.5 A	104 E	112 H	Brown	Black
Ames 15204	11.5 A	43.5 E	102 F	113 F	Light brown	Black
PI 654390	8.5 D	40 F	101.5 FG	112.5 FG	Yellow	Creamy yellow
HSD (0.05)	0.75	0.75	0.78	0.75		

Different letters within the same column indicate statistically significant differences at $P \leq 0.05$

Table 6: Mean values for biochemical attributes in 10 genotypes of amaranth over two years

Genotypes	*Chl a* (mg g^{-1} F. wt.)	*Chl b* (mg g^{-1} F. wt.)	Total *Chl* (mg g^{-1} F. wt.)	Carotenoids (mg g^{-1} F. wt.)	Seed protein contents (%)
PI 636194	0.035 A–C	0.098 A–C	0.133 A–C	3.16 A–D	18.12 A–C
Ames 1970	0.024 C	0.076 C–E	0.100 C–E	2.78 B–E	14.10 CD
PI 642733	0.044 A	0.116 A	0.160 A	3.40 A–C	13.16 D
PI 649305	0.029 BC	0.084 B–D	0.113 B–E	4.15 A	13.81 CD
PI 481272	0.037 AB	0.111 AB	0.148 AB	3.34 A–D	14.87 CD
PI 619265	0.034 A–C	0.091 A–D	0.125 A–D	3.80 AB	11.73 D
Ames 2031	0.025 BC	0.062 DE	0.087 DE	2.32 DE	15.06 B–D
Ames 18034	0.028 BC	0.079 C–E	0.107 C–E	2.77 C–E	16.18 A–D
Ames 15204	0.036 A–C	0.091 A–D	0.126 A–D	3.11 B–D	19.60 AB
PI 654390	0.029 BC	0.052 E	0.082 E	2.05 E	19.75 A
HSD (0.05)	0.013	0.03	0.04	1.03	1.25

Different letters within the same column indicate statistically significant differences at $P \leq 0.05$

panicle and seed colour (Table 5). Panicle colours of 'PI 636194', 'PI 649305', 'PI 481272' and 'PI 654390' were found yellow (Table 5). Genotype 'Ames 2031' and 'Ames 18034' showed brown panicle colour (Table 5); while 'Ames 15204' had light brown panicle colour. Genotype 'PI 642733', 'PI 619265' and 'Ames 1970' produced green, purple and dark pink colour panicles respectively (Table 5). Moreover, 'PI 481272', 'PI 654390', 'PI 642733', 'PI 619265' and 'Ames 1970' produced black colour seeds (Table 5).

Phenologial traits

Significant variations were observed among genotypes in terms of phenology (Table 5), genotypes initiated their bud growth between 40-48 days after emergence while genotypes reached milking stage 100-114 days after emergence (Table 5). Genotypes completed seed setting between 112 to 128 days. Genotypes 'PI 642733', 'PI 481272', 'PI 636194', 'PI 619265' were appeared as late maturing (Table 5).

Seed protein contents (%)

Significant ($P \leq 0.001$) genetic variations were noted among the genotypes for seed protein contents. Maximum value of seed protein was found in 'PI 654390' followed by 'Ames 15204', 'PI 636194' and 'Ames 18034' (Table 4). Minimum value of seed protein was recoded in 'PI 642733' (Table 6).

Leaf biochemical analysis

Significant variations were observed among genotypes in terms of leaf chlorophyll contents (Table 6); genotypes ‘PI 642733’, ‘PI 642733’, ‘PI 636194’ and ‘PI 619265’ had maximum leaf chlorophyll contents (*Chl a, Chl b* and Total *Chl*) while genotype ‘PI 654390’ had minimum leaf chlorophyll contents. Leaf carotenoid contents were also found different among amaranth genotypes (Table 6). Maximum leaf carotenoid contents were found in ‘PI 649305’ which was statistically at par with values of genotypes ‘PI 642733’, ‘PI 636194’ and ‘PI 481272’.

Discussion

In order to mitigate climate changes and malnutrition, FAO has selected some crops to be promoted and adopted in existing cropping systems and named these crops as *Future Smart Food* (Li and Siddique 2018). Amaranth is one of them. Therefore, for its introduction in Pakistan, its germplasm adaptability was explored in Faisalabad. Out for 10 genotypes, four genotypes were found good in terms of grain yield (Table 4). Amaranth grain yield ranged between 961.98-1280.69 kg $ha^{-1.}$ It showed that amaranth exhibits high degree of plasticity as earlier explained by Shukla *et al.* (2010). Phenotypic plasticity can be defined as ability of an individual to change its phenotype in response to variable environments (Fazlioglu and Bonser 2016). Due to this character this crop can be adapted in adverse and variable environments (Khanam and Oba 2014). These variations might be due to changes in weather conditions (Fig. 1) especially rise in temperature and increased day length during reproductive stages. In our study, it seemed that increased temperature influenced yield (Fig. 1) and genotypes experienced high temperature and showed different adaptability. Genotypes which produced high grain yield seemed more heat tolerant as well. Grain yield is a result of association and expression of many plant growth components and environmental conditions (Anjum *et al.* 2011). Abiotic stresses such as high temperature is also most important factor which restricts growth and productivity of major cereals (Umar 2006; Shwabe *et al.* 2013).

Furthermore, it is evident from time of seed setting (Table 5) that genotypes which were high yielder matured late, it can be linked to “stay green” character of genotypes; stay green character has been considered an important character for heat tolerance. It has also been observed that heat tolerant wheat genotypes had also high chlorophyll contents (Farooq *et al.* 2011) which is directly linked to stay green character. Moreover, adapted crop genotypes might also have good antioxidant system to detoxify toxic ROS which may otherwise be detrimental to macro molecules including chlorophylls (Hussain *et al.* 2018). The grain filling process is a reserve accumulation in developing grain which is sensitive to environmental factors which ultimately affect final seed yield (Yang and Zhang 2006). The most significant factor limiting seed yield is high temperature in cereals, high temperature induces shortening of vegetative phases, less light perception due to shortened life cycle and perturbation of carbon assimilation processes (photosynthesis, transpiration and respiration) (Stone and Basra 2001). So, it seems that low yielder genotypes were heat sensitive that’s why they matured earlier (Table 5). Heat stress induces metabolic changes especially excess ethylene production leading to early senescence and chlorophyll degradation. Thus, maintenance of stay green character is very important and considered as best indicator of thermo tolerance (Farooq *et al.* 2011). In our case study such type of results are evident (Table 6); genotypes which produced high grain yield had also more leaf chlorophyll contents (Table 6). Strong correlations were found between leaf chlorophyll contents and grain yield.

Phenological traits have key importance in crop production it directly influences the overall yield of a crop. In current study days taken to milk stage and days taken to seed setting were recorded. It was observed that genotypes reached at seed setting stage in 113 to 130 days after emergence, it seems large differences. Genotypes in which seed setting was late, might also took advantage of more grain filling period and produced grain yield. Adaptability of any genotype is influenced by environmental (as discussed earlier) and its own genetic factors. Like in cereals different yield contributing factor contribute final yield, so some plant characters of amaranth genotypes were also studied (Table 4) which might influence final grain yield. It is evident from Pearson correlation that haveir terminal panicles contribute to final grain yield.

For successful acclimatization of any crop in new environment, grain quality is also another important element which should not be neglected; therefore, grain protein contents were also measured in harvested grain of different genotypes. Grain protein contents ranged between 11.7 to 19.0% which is comparable with international published reports (Li and Siddique 2018).

Conclusion

Huge phenotypic plasticity was found among amaranth genotypes which were found beneficial to successful acclimatization of this crop in semiarid conditions of Faisalabad, Pakistan. Among the genotypes, maximum grain yield was produced by ‘PI 642733’ followed by ‘PI 619265’, ‘PI 636194’ and ‘Ames 15204’. This was linked with stay green character (more leaf chlorophyll contents) of genotypes for longer period, as depicted by more seed setting periods of high yielder genotypes. Furthermore, seed protein contents ranged between 11.7 to 19.0% which were comparable with already published report.

Author Contributions

Samreen Nazeer: Data analysis, initial draft, review and editing; Shahzad M.A. Basra: Conceptualization, supervision, review and editing; Shahid Iqbal: Conceptualization, supervision, review and editing;

Muhammad Bilal Hafeez: Methodology, review and editing; Ahmed Mateen: Data analysis using statistical package; Muhammad Zubair Akram: Methodology; Noreen Zahra: Methodology; review and editing; Shahbaz Khan: Review and editing; Jahanzaib: Methodology; Muhammad Sohail Saddiq: Formal analysis, review and editing.

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