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VARIABILITY STUDIES OFAFRICAN SPINACH (*Amaranthushybridus*) GERMPLASM IN BENUE STATE NIGERIA

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ABSTRACT

This work was carried out to assess the level of variability that exists among African spinach(*Amaranthushybridus*)collections.A total of sixty (60) accessions were collected from five (5) different markets within Makurdi town and planted in a Randomized Complete Block Design (RCBD) structure. Data was collected on fifty(50) characters (21 qualitative characters and 29 quantitative characters) andsubjected to Multivariate Statistical analysis on the Minitab 16.0 software.Resultsshowed a wide range of variability in most of the quantitative characters. Percentage germination ranged from 20 to 100%(mean = 51.97±3.83, CV=45.37%). Vigor index ranged from 27.50to 480.00 (mean =133.70 ± 15.90, CV% = 73.10%). Average number of spikes/plant and spike weight were 135.30 ± 38.60 and 35.70 ± 11.50 respectively.Number of branches, Spike weight and Number of spikes produced are the top three most variable characters based on CV values.Stem pigmentation and spike colorgave slight variation among qualitative characters. Accessions with good breeding qualities were selected. Correlation analysis showed relatedness and association among some of the character studied as number of seeds and number of spikes per plant were highly correlated (r=+0.98).Cluster analysis gave rise to 2 distinct groups with each group demonstrating similarities and differences in characters with a promise for use in cultivar development. The most divergent collections were found in the Wadata (AHWD1 and AHWD2) and Railway (AHRM3) markets. The information provided could be used by breeders while the highlighted distinct groups may serve as useful genetic resources.

Key words: *Amaranthushybridus*, Correlation, Improvement, Variability

1.0 INTRODUCTON

Amaranthus(family Amaranthaceae), collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants, consisting of approximately 60 species, which according to the uses for human consumption can be divided into grain and vegetable amaranths (Mlakar*et al*., 2010). The plant commonly called African Spinach is an annual herb or forb and as expected, the plant is in great abundance during the rainy season and it is one of the weeds of *amaranthus* species (Das, 2012) that is generally used as a leafy vegetable (Mobina and Jagatpati, 2015).*Amaranthushybridus*, a weed of great important particularly in Nigeria where it has been domesticated by almost all homes and has a long history of cultivation as a food crop that is still sometimes cultivated on a small scale for its edible leaves and seeds (TPD, 2019). It is a C4 plant that is widely distributed in the tropics (Ebert *et al*., 2011), a relatively drought-tolerant crop (Olufolaji*et al*., 2010) and as a leafy vegetable, it can be produced in regions and seasons where other crops are inaccessible, since they grow in poor soil conditions and tolerate temperatures up to 40ºC (Ebert *et al*., 2011).*A. hybridus* is a monoeciousplant having staminate and pistillate flowers on the same plant, it is self-fertilized as a means of reproduction, each type of flower has five (5) cream sepals and no petals, each staminate flower has five (5) stamens, while each pistillate flower has an ovary with three (3) styles, (Bayer, 2013) and the flowers are wind pollination. The plantis generally considered a weed in North America and is commonly known as smooth amaranth, though it is consumed as a staple food in other areas of the world such as Africa, Asia, the Caribbean and part of Europe (Abbasi*et al*., 2013).

One of the reasons why there has been recent interest in Amaranth is because of its nutritional qualities. The leaves, stems and heads of the plant are reportedly high in protein (15 – 24% on a dry matter basis) and the plant produces 30 – 60 t/ha of silage (80% moisture) (DAFF, 2010). Nutritional composition of this vegetable reveals high amount of protein, fat, fiber, ash, mineral elements, vitamins and amino acids (Mofunanya and Soonen, 2017). The Leaves of the plant contains high quality protein with relevant levels of lysine (Andine*et al*., 2013) and are used as leafy vegetables similar to spinach and can be made into tea that is purported to have astringent properties and to cure dysentery, diarrhea and ulcers (Paul *et al*., 2018), they possess

antibacterial effect, cleansing effect and also help to reduce tissue swelling (Singh and Sheoran, 2011) and hence, they have been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrisation. The plant constitutes a major part of the diet of people inthe middle and southern Nigeria where they areused mostly in the preparation of soup (Mofunanya and Sonnen, 2017)

Amaranth seed and amaranth seed oil is high in Vitamin E and squalene, which can be beneficial for people suffering from hypertension or cardiovascular disease (Anjal*et al*., 2013). Chemical composition analysis of the grains of the plant confirms their high potential for human nutraceutical uses (Das, 2016). As summed up by Fernard*et al*.(2012), grainsof amaranth are a healthy and nutritious food crop that could benefit people if it was produced and consumed in greater quantities.Morphological traits which are the phenotypic expression of genetic information are grouped into two: quantitative and qualitative traits, the latter is controlled by one or few genes while the former is controlled by interactions of many genes called polygenes (Aguoru*et al*., 2015). Characters interrelate to contribute to the overall function of plant and they may relate positively or negatively (Aguoru*et al*., 2015). The degree of relationship may be explored as morphological markers to monitor inheritance or likely appearance of other characters because hen two characters are closely related, improving one of the characters may affect the other character (Singh *et al*., 2016). The crop deserves attention in crop improvement programme and biotechnology to address threats to productivity. This work was carried out to assess the biotechnological and breeding values from variations that exist among African spinach (*Amaranthushybridus*) collections.

2.0 MATERIALS AND METHODS

2.1 Seed Collection

The seeds of African spinach(*Amaranthushybridus*) were obtained from three (3) different locations in five (5) different markets[North bank market (NB), Railway market (RM), Wurukum market (WK), Modern market (MM), Wadata market (WD)] within Makurdi town, Benue state.

2.2 Seed Identification and Coding

Seeds were identified and authenticated by Dr. J.O. Olasan of the Department of Botany, Federal University of Agriculture, Makurdi and were packaged in small white envelops which was appropriately in-scripted in order to remember the market of purchase as well as the locations within the markets.Codes were assigned to each of the accessions based on the place of collection and number of replications to ease identification of characters and the stress of continually writing the accession name and place of collection in full (Table 1).

2.3 Experimental Design and Treatment

A total of 60 accessions collected from 5 different markets locations within Makurdi town and planted. The Randomized Complete Block Design (RCBD) was used.

2.4 Site inspection and Preparation

An area was picked at the botanical garden site close to the school's water works due to its proximity to water for irrigation as the rains was yet to be stable and constant with due consideration given to soil type.The land was cleared and total of (15) ridges of 160cm long were made with an average height of 15cm from the ground to accommodate (4) accessions per ridge.

2.5 Planting

The seeds were planted per ridge and plant spacing of 28cm was allowed between the accessions.Sign post bearing all the accession codes were made out of wood and Polyvinyl chloride (PVC) material and placed against the accessions based on their order of randomization and planting in the field for easy identification.

2.6 Management practices

After planting, management practices wascarried out to ensure the effective growth of the plants. Watering of the plants was done on occasions when the rains were not forthcoming to prevent death of plants due to desiccation or water loss, weeding was done at regular intervals and ash was applied on the plants to prevent infestations by insects that feed on their leaves.

2.7 Data collection

A total of 50 characters were evaluated consisting of 21 qualitative characters that were assigned unique codes and values to indicate presence or absence (Table 2) and 29 quantitative characters (Table 3) that were assigned unique codes and their values were obtained using meter rule. Data collection on growth parameters commenced at day 15 after planting (DAP) and was done on a weekly basis till the 9^{th} week after planting.

2.8 Data Analysis

Data computation was done in an Excel work book and later imported into Minitab 16.0 software for Multivariate Statistical analysis. The relationships among the quantitative and qualitative characters were established using Pearson's Product Moment Correlation method. Cluster analysis was carried out using the Average Linkage method to generate a Dendrogram.

Table 1: Market Locations, Replicate and assigned codes to the Accessions.

Location	Replicates and Accession symbol
Modern market – 1	AHMM 1A, AHMM 1B, AHMM IC, AHMM ID
Modern market – 2	AHMM 2A, AHMM 2B, AHMM 2C, AHMM 2D
Modern market – 3	AHMM 3A, AHMM 3B, AHMM 3C, AHMM 3D
North bank market – 1	AHNB 1A, AHNB 1B, AHNB 1C, AHNB 1D
North bank market – 2	AHNB 2A, AHNB 2B, AHNB 2C, AHNB 2D
North bank market – 3	AHNB 3A, AHNB 3B, AHNB 3C, AHNB 3D
Wurukum market – 1	AHWK 1A, AHWK 1B, AHWK 1C, AHWK 1D
Wurukum market – 2	AHWK 2A, AHWK 2B, AHWK 2C, AHWK 2D
Wurukum market – 3	AHWK 3A, AHWK 3B, AHWK 3C, AHWK 3D
Wadata market – 1	AHWD 1A, AHWD 1B, AHWD 1C, AHWD 1D
Wadata market – 2	AHWD 2A, AHWD 2B, AHWD 2C, AHWD 2D
Wadata market – 3	AHWD 3A, AHWD 3B, AHWD 3C, AHWD 3D
Rail way market – 1	AHRM 1A, AHRM 1B, AHRM 1C, AHRM 1D
Rail way market – 2	AHRM 2A, AHRM 2B, AHRM 2C, AHRM 2D
Rail way market – 3	AHRM 3A, AHRM 3B, AHRM 3C, AHRM 3D

Table 2: Qualitative Characters Evaluated and their assigned codes and values

S/No	Symbols	Qualitative Characters	Codes
1	PHB	Plant habit	1 = Annual, 2 = Biennial
2	P	Phyllotaxy	1 = Opposite, 2 = Alternate
3	LS	Leaf shape	1 = Entire, 2 = Serrate
4	LM	Leaf margin	1 = Entire
5	SP	Stem pigmentation	1 = Reddish stem, 0 = Greenish stem
6	SPS	Stem hairiness/ pubescence	1 = Pubescence, 2 = No pubescence
7	LPS	Leaf hairiness/ pubescence	1 = Pubescence, 2 = N0 pubescence
8	SLC	Sepal colour	1 = Cream, 2 = Red
9	LT	Leaflet texture at days 60	1 = Soft, 0 = Hard
10	FS	Floral symmetry	1 =Bilateral/Actinomorphic, 2 = Radial/Zygomorphic
11	ST	Stamen type	1 = United
12	AF	Anther fixation	1 = Free/Dithecous
13	PT	Placentation type	1 = Basal placentation
14	WA	Whorl adhesion	0 = No adhesion, 1 = Adhesion present
15	WC	Whorl cohesion	0 = No cohesion, 1 = Cohesion present
16	PS	Petal shape	0 = No petal
17	FF	Floral formula	1 = $\mathrm{Br\,Brl}\oplus 0\nearrow_{+}\ \mathrm{P_{4\text{-}5}\,A_{4\text{-}5}C_{(2\text{-}3)}}$
18	SPC	Spike colour	1 = Green, 2 = Green & Red, 3 = Red
19	SDC	Seed colour	1 = Blackish Red
20	ST	Seed type	1 = Dry, Utricle
21	SHT	Shell texture	1 = Soft, 2 = Hard, 3 = Very hard

Table 3: Quantitative Characters Evaluated and their units of measurement

S/No	Symbols	Quantitative characters	Units of measurement
1	DOG	Day of germination	
2	NOG	Number of germination	Head count
3	%G	Percentage germination	%
4	PS	Plant spread	cm
5	VI	Vigor index (percentage germination × seedlings length)	
6	DOM	Day of maturation	
7	IL	Internode length	cm
8	NOSP	Number of spikes	Head count
9	FL	Flower length (Calculated as pedicel length + length of floral whorl)	cm
10	NOS	Number of sepal per flower	Head count
11	NOP	Number of petal per flower	Head count
12	NOB@30	Number of branches at day 30	Head count
13	NOB@60	Number of branches at day 60	Head count
14	PH@30	Plant height at 30	cm
15	PH@60	Plant height at 60	cm
16	LL	Leaf length	cm
17	LW	Leaf width	cm
18	WLW	Wet leaf weight	g
19	LPL	Leaf petiole length	cm
20	SC	Stem circumference	cm
21	NOS/P	Number of spikes/plant	Head count
22	SL	Spike length	cm
23	SW	Spike width	cm
24	SWT	Spike weight	g
25	NOSD/P	Number of seeds/ plant	Head count
26	NOSD/S	Number of seeds/spike	Head count
27	SDL	Seed length	cm
28	SDWT	Seed width	cm
29	SDW	Seed weight	g

3.0 RESULTS AND DISSCUSSION

A total of 38 accessions germinated while 22 accessions did not germinate out of the 60 accessions planted. The highest number of germination was observed in the accession (AHWD3) whileaccession AHNB1,AHRM3 andAHMM1 had the lowest numbers of germination. Descriptive statistics of the quantitative characters studied are shown in table 4. Day of germination had a mean of 4.74± 0.35, CV% of 44.90 andranged from day 2 to day 10. Earliest germination was recorded on day 2 while delayed germination was recorded on day 10. Percentage germination ranged from 20- 100%, CV% of 45.37 and a mean value of 51.97±3.83. Low percentage germination (%G) ranged between 0-40% while high percentage germination (%G) ranged between 40%-100%. At day 30, Accession AHMM2A had the minimum plant height of 3cm while the maximum plant height of 49cm was recorded in the accessionAHWD1C. Accessions AHWD3B and AHRM1Chad the minimum leaf length of 5cm each, while the maximum leaf length was recorded in accession AHRM2D) to be 23.5cm. Accessions AHRM1C, AHMM2A and AHNB2A had the least leaf width of 2cm eachwhile the highest leaf width of 21.5cm was recorded was in the accession AHWD2C. The lowest wet leaf weight of 0.6g was recorded in the accession AHNB3D while the highest wet leaf weight of 7.14g was recorded in the accession AHRM2A. The lowest recorded value of 1.3cm for Leaf petiole length was in the accession AHMM1D while the accession AHWK2Chad the highest value of 18.5cm.Accession AHMM2A had the lowest stem circumference of 0.6cm while the highest was recorded in the accession AHRM2B to be 14cm. Lowest Spike width of 1.2cm was recorded in the accessions AHWD1C) and AHRM1B while the highest spike width of 15cm was recorded in the accession AHWK2D. Qualitative characters studied showed Plant habit, phyllotaxy, leaf shape, leaf margin, stem hairiness, leaf hairiness, leaflet texture, floral symmetry, stamen type, anther fixation, placentation type, whorl adhesion, whorl cohesion, floral formula, seed color, fruit type and shell texture were all the same in all the accessions except slight variations recorded in Stem pigmentation and spike color (Table 5).

Pearson's correlation matrix (Table 6) of some selected quantitative characters showed that the highest correlation was between Number of seeds/plant (NOSD/P) and Number of spikes/plant (NOS/P) (R = 0.98). Number of seeds/spikes highly correlated with seed weight (R = 0.90), Plant height at day 60 highly correlated with leaf length (r= 0.75), Percentage germination had a moderate correlation with plant spread. The results of cluster analysis as represented by the dendrogramwhich is based on similarity coefficient divided the accessions into two main clusters (cluster 1 and cluster 2) with the level of

similarities between the clusters ranging from 99.88 to 70.28 indicating that the accessions studied were similar with little variability existing among them (Figure 1). The most divergent collections were found in the Wadata (AHWD1 and AHWD2) and Railway (AHRM3) markets.

The value of the African spinach is now being recognized, as evident by the rapid expansion in its production in different parts of the world. It is a cheap source of nutrients and antioxidants, easy to grow, and adapted to local growing conditions. Its potential as an alternative source of food is becoming popular because of the increasing demand for healthy foods in the market. A detailed breeding and taxonomical knowledge will allow farmers to produce more improved accessions allowing its conservation through use.The broad variability observed in spike related traits is valuable from the viewpoint of the genetic improvement of *Amaranthushybridus*. Wu *et al*. (2000) in a study of 229 genotypes from 20 *Amaranthus* species reported wide variability which was useful in cultivar improvement for agronomic traits, a finding that aligned with the work done byVaralaskshmi (2004).

Correlation measures the intensity of association between variables and breeding for yield would be very effective when there is a positive association among characters. In this study,strong positive correlation was observed between many pairs of characters such as between leaf morphology and seed morphology. These findings can be exploited directly or indirectly for the genetic improvement of the amaranths as suggested by Hazra and Basu, (2000). Furthermore, Number of seeds per plant depend greatly on number of spikes per plant which signifies that if breeders can genetically improve the genes responsible for spike production, seed production will be greatly and indirectly improved, percentage germination had no significant effect on spike length so breeders who are not after speed or rate of germination can work on accessions with great spike length for improvement, the negative correlation between leaf length and number of seeds produced per spike could also be useful as farmers could harvest vegetables for a long period before harvesting grains especially if the breeding line could be developed that would combine high leaf and grain yield concurrently.

The results from cluster analysis in this study which indicated a very small degree of variability existing among the accessions studied is in tandem with the findings of Oboh (2007) who reported that a great degree of variability exist among the Nigerian accession of *Amaranthushybridus*. This work goes further to prove the existing report that variability among *Amaranthus* genus is based on anatomical and morphological differences that raise the possibility of further subgenera division (Costea*et al*., 2001).

Table 4: Descriptive Statistics of Quantitative Characters studied

Variables	Mean	SE Mean	(CV%)	Minimum Range	Maximum Range
Day of germination	4.74	0.35	44.90	2.00	10.00
Number of germination	2.18	0.16	44.98	1.00	4.00
Percentage germination (%)	51.97	3.83	45.37	20.00	100.00
Plant spread(cm)	3.63	0.31	51.79	0.90	8.00
Vigor index(cm)	133.70	15.90	73.10	27.50	480.00
Internode length(cm)	1.62	0.14	51.14	0.30	3.10
Number of spikes	130.00	39.30	183.84	0.00	917.00
Flower length(cm)	9.40	1.87	122.73	0.00	40.00
Number of branches at day 30	1.11	0.35	192.55	0.00	7.00
Number of branches at day 60	2.42	0.81	206.55	0.00	19.00
Plant height at day 30(cm)	16.91	1.56	56.89	3.00	49.00
Plant height at day 60(cm)	68.83	5.71	51.15	17.00	154.00
Leaf length(cm)	15.15	0.99	40.20	5.00	23.50
Leaf width(cm)	8.04	0.74	56.92	2.00	21.50
Wet leaf weight(g)	2.37	0.35	90.27	0.09	8.96
Leaf petiole length(cm)	8.50	0.76	55.45	1.00	18.50
Stem circumference(cm)	6.68	0.52	48.30	0.60	14.00
Number of spikes/plant	135.30	38.60	175.89	0.00	917.00
Spike length(cm)	9.40	1.87	122.73	0.00	40.00
Spike weight(g)	35.70	11.50	198.41	0.00	354.00
Spike width(cm)	3.80	0.71	115.21	0.00	15.00

Table 5: Data on all the qualitative characters

Characters	Numbers of accessions present
Plant habit	Annual
Phyllotaxy	Alternate
Leaf shape	Entire
Leaf margin	Entire
Stem pigmentation	37 Green, 1 Red
Stem hairiness	Pubescence
Leaf hairiness	Pubescence
Leaflet texture	Soft

Floral symmentry	Bilateral/Actinomorphic
Stamen type	United
Anther fixation	Free/Dithecous
Placentation type	Basal
Whorl adhesion	Absent
Whorl cohesion	Present
Floral formula	Br Brl⊕ $0^{\nearrow}_{+}$ $P_{4\text{-}5}A_{4\text{-}5}C_{(2\text{-}3)}$
Spike colour	37 Green, 1 Red and Green
Seed colour	Reddish black
Fruit type	Dry/Utricle
Shell texture	Hard

Table 6: Correlation Matrix of Selected Quantitative Character Traits

Characters	**%G**	**PS**	**VI**	**NOB@60**	**PH@60**	**LL**	**WLW**	**NOS/P**	**SL**	**NOSD/S**	**NOSD/P**
PS	0.46										
VI	0.53	0.19									
NOB@60	-0.05	-0.02	0.04								
PH@60	-0.07	0.28	0.33	0.41							
LL	-0.06	0.08	0.40	0.19	0.75						
WLW	-0.02	0.16	0.36	-0.08	0.60	0.53					
NOS/P	0.08	0.13	-0.04	0.25	0.35	0.20	-0.07				
SL	-0.00	0.24	-0.14	0.46	0.47	0.01	-0.00	0.67			
NOSD/S	-0.09	0.06	-0.20	0.05	0.15	-0.19	-0.16	0.51	0.67		
NOSD/P	0.08	0.22	-0.07	0.25	0.37	0.18	-0.09	0.98	0.72	0.55	
SDWT	-0.13	-0.19	-0.19	0.07	0.07	-0.20	-0.20	0.41	0.60	0.90	0.40

*Significant at <0.05 level of probability, PS = Plant spread, VI = Vigor index, NOB@60 = Number of branches at day 60, PH@60 = Plant height at day 60, LL = Leaf length, WLW = Wet leaf weight, NOS/P = Number of spike/plant, SL = Spike length, NOSD/S = Number of seeds/spike, NOSD/P = Number of seeds/plant, SDWT = seed weight.

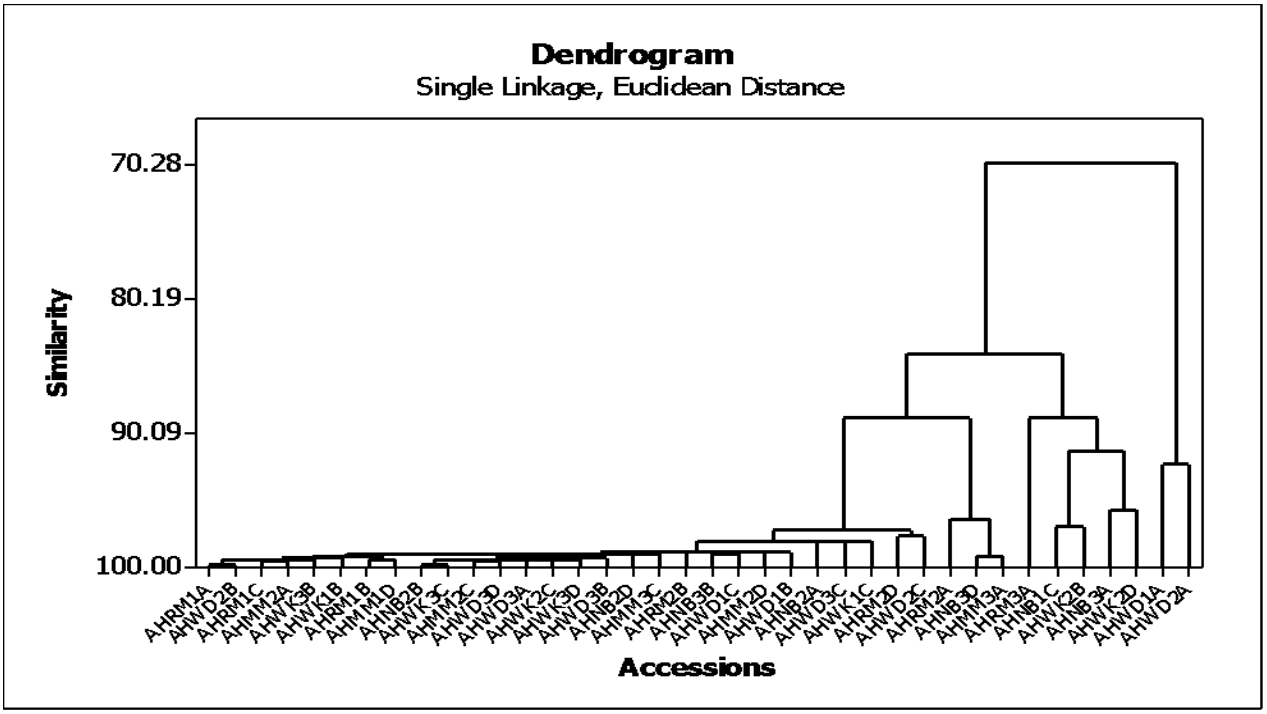

Figure 1: Cluster Analysis Dendrogram of 60 Accessions of*A. hybridus.*

4.0 CONCLUSION

Results showed a wide range of variability in most of the quantitative characters assessed Number of branches, Spike weight and Number of spikes are the top three most variable characters. Stem pigmentation and spike color gave slight variation among qualitative characters. The most divergent collections were found in the Wadata (AHWD1 and AHWD2) and Railway (AHRM3) markets. The distinct groups could serve as breeding lines for genetic improvement of the plant through cross-breeding. Thus, the observed variability could be explored in the biotechnological research of the crop in the overall goal of food security.

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