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Hybridization Studies of Okra (*Abelmoschus spp.* (L.) Moench) Accessions

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Abstract *— Okra (Abelmoschus spp. L. Moench) is an important multi-purpose vegetable crop cultivated and consumed across all tropical and temperate regions of the world. In Ghana, it is popular in all ten regions and increasing quantities are exported to Europe in the fresh form. The crop has received little attention by way of breeding to produce varieties combining the most desirable qualities to boost local cultivation and export. Ten accessions of Abelmoschus spp., comprising two species, A. esculentus (T1, T2, T3, VT, ID and AG) and A. callei (KB, AM, YL and T4) collected from six geographical regions of Ghana were crossed in all possible combinations to assess inter-specific as well as intra-specific hybridization between and within species. All six accessions of Abelmoschus esculentus were able to hybridize with one another in both direct and reciprocal cross combinations with high degree of crossability index (CI) (45.71% to 90.32%). On the other hand, cross-compatibility among A. esculentus and A. callei was successful only in one direction when A. esculentus was used as females also with a CI between 34.48% and 60%. Parental lines T3 and T1 emerged as the most compatible female and male.*

Keywords — *Okra, Accessions, Hybridization, Inter-specific, Intra-specific, crossability*

I. INTRODUCTION

Artificial sexual hybridization is a conventional breeding approach which involves crossing of selected genotypes in order to introgress genes, to introduce new genetic variability for generating new or novel varieties [1]. This is usually aimed at incorporating genes of desirable traits such as disease resistance and high yield in one genotype into the genetic background of the other genotype to produce superior hybrids. It is a very useful approach in quantitative genetic analysis such as studies of combining ability and expression of heterosis towards improvement of targeted traits. In cross-pollinated species such as sweet potato, the phenomenon of self-incompatibility and high levels of cross-compatibility promotes high rates of cross-fertilization after hand pollination [2, 3].

However, due to genetic and reproductive barriers such as homogamy in which synchronization of anther dehiscence, stigma receptivity or close proximity of stigmas with anthers and self-pollinated crops generally exhibit very low cross-fertilization rates after artificial pollination [4, 5]. Differences in genetic and floral morphology such as the effects of heterostyly— different style length among genotypes utilized as parents— have been identified to contribute to the low success rates after artificial pollination [6, 7, 8]. For instance in cassava, the genotype of the female plays prominent role in determining success of the crosses than the pollen source [9]. Apart from floral and genetic factors, environmental conditions particularly low temperature and high humidity are also known to play very important roles in determining hybridization success after hand pollination [3, 10]. As a result, assessment of crossability relationships among genotypes of self-pollinating crops such as okra is a first step for systematic and effective planning of crop improvement programmes through artificial hybridization in order to efficiently utilize genetic diversity [6].

The major problem underlying okra productivity is low yielding potential of current varieties and reduction in yield due to frequent infestation of pests and diseases, especially the fruit and shoot borer and Yellow Vein and Mosaic Virus (YVMV) [11], as well as the lack of improvement on the well adapted local landraces. YVMV transmitted by white fly (*Bemisia tabaci*) is the main limiting factor in cultivation affecting fruit yield, causing a loss of 50 to 95 per cent depending on the stage of the crop growth at which infection occurs [12] and fruit quality of okra. Since the disease cannot be controlled by chemical means, the only practical solution to this problem is to develop tolerant and resistant varieties [13]. Interspecific hybridization is considered a possible mechanism of plant diversification. Interspecific hybridization followed by backcrossing and selection in the segregating generations is an effective method for developing high yielding disease resistant varieties. Hybridization including wild and cultivated species has long been used for transfer of genetic material in crops. A promising breeding method for creation of new variability is wild hybridization that became a more common practice after the advancement of hybridization techniques [14]. Wild relatives of crops have been recognized as an important source of useful traits for breeding programmes. Also, yield levels have been improved substantially through intensive and concerted breeding efforts, and as further yield advances seem to be more difficult necessitating the application of newer breeding approaches. The required goal of increasing productivity in the quickest possible time can be achieved only through heterosis breeding.

It is, therefore, important to enhance productivity of the local landrace varieties of okra through breeding to boost vegetable farmers’ interest in large-scale cultivation of the crop and for adoption by Ghanaian and export market. Intra-specific and inter-specific hybridization among locally adapted cultivars may therefore play a very important role in producing a broad-based segregating population from which recurrent selection could be carried out to obtain hybrids which combine high yield and disease resistance with other desirable traits such as high nutritional and anthocyanin contents, as well as fruit characteristics required for export.

This study seeks to investigate crossability through inter-specific and intra-specific hybridization of okra from cultivars assembled from different parts of the country. The main objective of this study was to investigate crossability relationships among ten local landraces and exotic cultivars of okra for key yield-determining traits in order to obtain relevant information on their breeding behaviour for use in future breeding programmes towards improvement of landraces in Ghana.

II. MATERIALS AND METHODS

A. Location and edaphic conditions of the experimental site

The experiment was carried out at the research farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission (GAEC) at Kwabenya, near Accra. The experimental site is located at 05° 40' N and longitude 0° 13' W at an elevation of 76 m above sea level within the coastal savanna agro-ecological zone. The soil at site belongs to the Nyigbenya-Haatso series, which is typically well drained savanna Ochrosol (Ferric Acrisol), derived from quartzite and Schist [15].

B. Germplasm Assembly

Twelve accessions of okra were collected from six geographic regions of Ghana (Eastern, Volta, Ashanti, Greater-Accra, Upper East and Brong-Ahafo regions) (Table 1) and used as parents to produce F1 hybrids.

TABLE 1:
Okra Accessions used in the Study

Accession code	Origin	Accession name	Accession owner
T1 *(A. esculentus)*	Upper East region	Yire marna 1	Amitaaba
T2 *(A. esculentus)*	Upper East region	Yire marna 2	Amitaaba
T3 *(A. esculentus)*	Upper East region	Yire marna 3	Amitaaba
T4 *(A. caillei)*	Upper East region	Yire marna 4	Amitaaba
AM *(A. caillei)*	Eastern region	Amanfrom	Ahiakpa
VT *(A. esculentus)*	Volta region	Volta	Ahiakpa
ID *(A. esculentus)*	Greater Accra region	Indiana	Ahiakpa
AG *(A. esculentus)*	Ashanti region	Agric Short	Ahiakpa
YL *(A. caillei)*	Brong-Ahafo region	Yeji Local	Ahiakpa
KG *(A. caillei)*	Ashanti region	Kortebotor green	Ahiakpa
KB *(A. caillei)*	Ashanti region	Kortebotor pink	Ahiakpa
CSL *(A. esculentus)*	GreaterAccra region	Clemsin spineless	Agrimat Ghana

C. Land Preparation and Experimental Design

A total land area of 45 m x 30 m was acquired and cleared; all stumps were removed and ploughed to a fine tilth for planting. Single row planting was used with four replications, each replicate measuring 17.5 m x 12 m, and separated by a distance of 2 m from the other. All rows within the various replications were randomly assigned by drawing lots to avoid bias and each plot was labelled.

D. Seed Sowing and Field Management Practices

Seeds of the various accessions were sown after a heavy rainfall that facilitated uptake of water by the seeds for germination. The seeds were sown at a depth of 2 cm, at a spacing of 1 m x 0.70 m between and within rows to allow free movement during emasculation, with three to four seeds per hill and thinned to two per hill after germination. No fertilizer was applied, but weeds were controlled and other agronomic and management practices were carried out. Weeding was done fortnightly. The rainfall pattern during this period was not quite consistent and regular hence there was the need for supplementary water for irrigation to support plant growth and development.

E. Emasculation and crossing operation

Tools used for the emasculation process were, a pair of forceps, 70 % alcohol, cotton wool, a stapler, and paper envelops. Five healthy plants of each accession were selected to serve as parents for hybridization. Emasculation was carried out prior to pollination by first identifying a matured bud as shown in step one. Next, the calyx of the matured bud was cut open to expose the corolla. The corolla was removed by cutting it close to the receptacle to expose both the androecium and gynoecium without damaging the fruit bud. The anthers were then removed to feminize the hermaphroditic flowers. Finally, the calyx was folded back and sealed in paper envelops to prevent insect and self-pollination.

Pollination was performed the next morning from 6:00 am to 10:00 am by dusting the receptive stigma of the emasculated flower with pollen from selected male parents. For each cross, 20 flowers were pollinated and reciprocal crosses were also made. Immediately after emasculation and pollination, the flower buds were covered with paper bags and clipped to prevent contamination from undesirable pollen and properly tagged in order to identify the pollen donor and recipient as well as the time that each particular cross was made. Between successive emasculation of flowers belonging to different accessions, the pair of forceps was sterilized with alcohol to prevent contamination with pollen from earlier sources. Three days after pollination, the paper bags were removed to allow the fruits to develop properly.

F. Evaluation of Hybridization Success

Success of hybridization was assessed through observation of each flower three days after pollination. Fertilized flowers developed fruit capsules with white seeds between 3 to 4 days after pollination depending on the accessions crossed and where fertilization failed, the flowers dropped 2 to 3 days after pollination without developing any fruit capsules.

G. Estimation of Hybridization Success

Fruit set percentage of each cross was calculated according the formula of Nunekpeku et al. [16].

$$FS\ (\%) = \frac{NFF}{NFP} \times 100$$

Fruit set percentage = (FS); Number of fruit formed = (NFF); Number of flowers pollinated = (NFP)

H. Estimation of crossability index

The crossability index was estimated using the following formula [16].

$$CI = \frac{(\Sigma\ FS\ F1)}{(\Sigma\ FS\ P1 + FS\ P2)\ /2)} \times 100$$

Where, CI (%) = Crossability index; FS F1 = fruit set for F1 hybrid; FS P1 = fruit set for parent one; FS P2 = fruit set for parent two.

III. RESULTS

Results of the crossability success among 12 accessions okra, five *A. caillei* T4, KG, KP, YL and AM and seven accessions of *Abelmoschus esculentus* T1, T2, T3, CSL, VT, ID and AG in inter-specific and intra-specific hybridization using reciprocal crosses is presented in Table 2.

TABLE 2:
Cross Compatibility Success among 12 Accessions of *Abelmoschus esculentus* and *caillei*

♂	T1	T2	T3	VT	ID	AG	CL	T4	AM	YL	KB	KG
♀												
T1	X	√	√	√	√	√	*	√	√	√	√	N
T2	√	X	√	√	√	√	*	√	√	√	√	N
T3	√	√	X	√	√	√	*	√	√	√	√	N
VT	√	√	√	X	√	√	*	√	√	√	√	N
ID	√	√	√	√	X	√	*	√	√	√	√	N
AG	√	√	√	√	√	X	*	√	√	√	√	N
CL	*	*	*	*	*	*	X	*	*	*	*	*
T4	N	N	N	N	N	N	*	X	N	N	N	N
AM	N	N	N	N	N	N	*	N	X	N	N	N
YL(♀)	N	N	N	N	N	N	*	N	N	X	N	N
KB(♀)	N	N	N	N	N	N	*	N	N	N	X	N
KG(♀)	N	N	N	N	N	N	*	N	N	N	N	X

♂= Male Parent; ♀= Female Parent; √ = Successful cross; X = Self; N = Not successful; * = Cross not carried out.

Successful hybridization was obtained only in one direction of direct crosses and not reciprocal crosses between *A. esculentus* cultivars and *A. caillei* in this study. Successful hybridization between *A. esculentus* when used as female (♀) and *A. caillei as male* (♂) was achieved, all attempted reciprocal crosses failed to develop fruit capsules and dropped off two or three days after pollination. This was partly due to poor synchronization of flowering, and inability of some of the accessions to produce enough flowers to complete reciprocal crosses among the accessions of *Abelmoschus caillei*. However, hybridization attempts among accessions belonging to *Abelmoschus esculentus* mating group, was successful though to varying levels in different cross combinations. Accessions T1, T2, T3, ID, VT and AG emerged as most compatible female and male parents, yielding 30 successful hybrids in reciprocal crosses.

On the contrary, direct and reciprocal crosses (whether used as male or female) among all six accessions of *Abelmoschus esculentus* were all successful (Table 3).

TABLE 3:
Hybridization Success (%) among Six Accessions of *Abelmoschus esculentus* in Pairwise Crosses

♂	T1	T2	T3	VT	ID	AG
♀						
T1	X	√	√	√	√	√
T2	√	X	√	√	√	√
T3	√	√	X	√	√	√
VT	√	√	√	X	√	√
ID	√	√	√	√	X	√
AG	√	√	√	√	√	X

♂= Male Parent; ♀= Female Parent; √ = Successful cross.

Twenty flowers were selfed for all the accessions and all were successful. The percent fruit set, average number of seeds per fruit and percent germination among parental accessions okra (*Abelmoschus esculentus*) are shown in Table 4. T2 and VT gave the highest values (95%) and YL the least value (60%) for percentage fruit set percentage. Accessions VT and T3 (69 and 25) gave the highest and least value respectively for average number of seeds per pod. Accessions VT and YL recorded the highest and lowest values for germination percentage respectively (Table 4).

TABLE 4:
Percent Fruit Set, Average Number of Seeds per Fruit and Percent Germination among Parental Accessions Okra (*Abelmoschus esculentus*)

Parental accession	NFC	FS (%)	ANSP	CI (%)
T1	20	80	48	90
T2	20	95	39	80
T3	20	75	25	85
T4	20	70	54	70
VT	20	**95**	**69**	**95**
ID	20	75	32	90
AM	20	**95**	65	90
KB	20	70	68	70
YL	20	60	38	65
AG	20	75	59	80

Bolded and underlined values represent respectively highest and lowest percentage fruit set (FS %), number of flowers crossed (NFC), average number of seeds per pod (ANSP) and percentage crossability index (CI %).

The results obtained for percentage fruit set; average number of seeds per pod, percentage germination and crossability index of 29 F1s obtained from intra-specific hybridization shown in Table 5. VT X T1 gave the highest values (75%), while T3 X ID, ID X VT and T1 X AG scored the least value (40%) for percentage fruit set. For average number of seeds per pod, VT X T2 (48) and T3 X ID (16) gave the highest and least value respectively. In all, VT X T3 recorded the highest value (90.00%) and ID X AG the least (30%) for percentage germination, while crossability index values ranged between 90.32% and 45.71% for the crosses between T3 X T1 and T1 X AG respectively.

Table 5 displays the percentage fruit set; average number of seeds per pod, percentage germination and crossability index of 23 F1s obtained from intra-specific hybridization. Twenty flowers were crossed for accessions. The cross VT X AM gave the highest value of (50%), while the crosses T3 X KB and ID X KB scored the least value of (25%) for fruit set percentages. While for average number of seeds per pod, the crosses VT X AM and ID X YL gave the highest and least value of 43 and 6 seeds respectively. For germination percentage, the cross ID X KB recorded the highest value of (85%) while, the cross AG X YL gave the least value (10%). With respect to crossability index, the cross T3 X AM exhibited the highest crossability of (60.00%) while the crosses T3 X KB and ID X KB gave the least crossability index of (34.48%).

TABLE 5:
Percentage Fruit Set; Average Number of Seeds per Pod, Percentage Germination and Crossability Index of 29 F1s obtained from Intra-Specific Hybridization

OFFSPRING	NFC	FS (%)	ANSP	G (%)	CI (%)
T2 X T1	20	65	35	70	74.29
T3 X T1	20	70	44	60	**90.32**
VT X T1	20	**75**	47	65	85.71
AG X T1	20	65	45	75	74.29
T1 X T2	20	55	43	85	62.86
T3 X T2	20	70	40	80	82.35
ID X T2	20	45	28	75	52.94
VT X T2	20	70	**48**	60	73.68
AG X T2	20	60	46	75	63.16
T1 X T3	20	55	37	80	70.97
T2 X T3	20	65	43	75	76.47
ID X T3	20	50	20	60	66.67
VT X T3	20	65	22	**90**	76.45
AG X T3	20	55	20	75	64.71
T1 X ID	20	45	20	55	58.06
T2 X ID	20	60	18	55	70.59
T3 X ID	20	40	16	63	53.33
VT X ID	20	60	34	60	70.59
AG X ID	20	50	26	70	58.82
T1 X VT	20	60	25	65	77.42
T2 X VT	20	55	31	75	58.89
T3 X VT	20	50	23	75	58.82
ID X VT	20	40	27	52	47.06
AG X VT	20	60	45	70	63.16
T1 X AG	20	40	41	60	45.71
T2 X AG	20	45	40	70	47.35
T3 X AG	20	60	30	50	70.59
ID X AG	20	45	29	30	52.94
VT X AG	20	70	36	75	73.68

Bolded and underlined values represent respectively highest and lowest fruit set percentages FS (%), average number of seeds pod (ANSP), percentage seed germination G (%) and crossability index CI (%).

IV. DISCUSSIONS

A. Cross compatibility studies among accessions of A. caillei and A. esculentus

Results of the investigation revealed that all six accessions of *A. esculentus* utilized in this study are compatible with one another in both direct and reciprocal crosses, a strong indication of no major crossability barriers among genotypes of this sub-species of okra. Highest fruit set, average number of seeds per crossed fruits and highest crossability index were observed. However, crosses between *A. caillei* and *A. esculentus* were successful only when *A. esculentus* was used as female and *A. caillei* as male. Sheela [17] stated that reciprocal differences in compatibility of the two species exist. In contrast, successful crossing between these two species was also reported by Hamon and Hamon [18], where highest fruit set, average number of seeds per crossed fruits and highest crossability index were observed when *A. caillei* was used as female parent.

Accessions T3 and T1 were the most compatible female and male parents with the greatest CI (90.32%) value. This shows their inherent potential as pollen donor and recipient respectively, since all accessions were crossed under the same conditions. Hence, T3 and T1 would be suitable maternal and paternal parents respectively to cross with the other accessions in future breeding of okra in Ghana through intra-specific hybridization. T4, AM, KB and YL also failed to register any success as female parents, thus could be utilized only as paternal parents (pollen donors) in breeding programmes. Similar results were reported by Nunekpeku et al. [16] who studied crossability in cassava and apple respectively.

B. Hybridization success among accessions of A. caillei and A. esculentus in pairwise crosses

Results of the investigation reveal that hybridization success of all accessions of *A. esculentus* utilized in the study were high with percentage fruit set ranging from (40% to 75%), which indicates high fertility rates of the genotypes of this sub-species of okra. In line with observations of Valdiani et al. [6] as well as De-Block and Igersheim [8]. On the other hand, hybridization success of crosses among accessions of *A. caillei and A. esculentus* was generally in one direction only - when *A. caillei* was used as male parent. Accessions involving ID as female and T1 as male produced shrivelled seeds which failed to germinate – a sign of hybrid inviability. Major causes of hybrid inviability might be due to non-compatibility of the parental chromosomes, cytoplasmic genic interactions and non-compatibility between embryo and the surrounding tissue called somaplastic sterility [19]. Crosses which produced shrivelled and non-viable seeds indicate involvement of post-zygotic sterility during inter-specific hybridization. Similar results were obtained by Sheela [17]. In this incompatibility, zygote formation might be prevented by failure or ineffectiveness of pollen growth or failure of fertilization [20]. However, viable hybrids were achieved through embryo culture technique, by Gadwal et al. [21]. Genetic relationships among genotypes are known to play an important role in determining hybridization success after artificial pollination [7, 22, 23].

Consequently, genetic difference among the accessions of *A. caillei* and *A. esculentus* in this study could have also contributed to the observed variations in reciprocal crosses. From the present findings, it was observed that highest percentage fruit set, number of seeds per crossed fruit, percentage germination were achieved when VT was used as female and highest crossability index obtained when T3 was used as female parent while crossing with T1, T2, T3 and T1 respectively. This is in line with findings of Jambhale [24]. However, Sheela [17] reported that reciprocal crosses registered higher compatibility than the direct crosses, while Cheriyan [25] observed no differences between direct and reciprocal crosses involving *A. esculentus* and *A. manihot*. Lowest fruit set was observed in the inter-specific hybridization betweenT3 X KB and ID X KB. Also, ID X YL registered the least value for average number of seeds per fruit and least crossability index was observed in the cross between AG X YL. Low seed set and recovery of shrivelled seeds may be due to partial or complete failure of the endosperm owing to genetic imbalance.

V. CONCLUSIONS

Outcomes of crossability studies help breeders to determine appropriate strategies or breeding designs to adopt to transfer genes of desirable traits present in one genotype into the genetic background of other genotypes to produce novel varieties. Where genotypes are compatible with one another in both direct and reciprocal crosses as was achieved for accessions of *A. esculentus* in this study, a full diallele analysis can be carried out. However, where complete pairwise crosses are not achieved as it came out for accessions of *A. caillei* and *A. esculentus*, partial diallele or other breeding designs where genotypes can be used as either male or female parents only would be suitable.

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