A Comparative Study of Emasculation Techniques in *(Abelmoschus esculentus* L. Moench) and *Abelmoschus caillei* (A. Chev.) Steveis in Relation to Heterostyly and Crop Season

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Abstract

To achieve rapid transfer of genes from the West African tall okra (late okra) Abelmoschus caillei (A.Chev) Stevels. to the conventional okra (early okra), Abelmoschus esculentus(L.)Moench, the well known Destructive Emasculation Technique (DET) in okra was modified while a new technique, Non Destructive Emasculation Technique (NDET) was developed which was found most ideal for sensitive okra cultivars especially during the dry periods of the year. When the two emasculation techniques were compared for early and late okra for both the rainy and dry seasons, the results showed that success with NDET was significantly higher than with DET for both okra types for the two seasons but there were no significant differences in the seasonal responses of the two okra types to the two emasculation techniques. Also the response of the two okra types to the DET showed that the response of late okra was better than that of early okra for the two seasons. The results further confirm the more rugged nature of late okra when compared with conventional okra. Three types of heterostyly; "Epistigmatic", "Peristigmatic" and "Hypostigmatic" conditions were described for the first time in okra. The type of heterostyly exhibited determined whether the Up-Down or Down-Up method of anther removal was adopted for the NDET. NDET is recommended for emasculation of okra as well as other crops in the Malvaceae family in the West African region.

Keywords: Okra, *Abelmoschus esculentus - Abelmoschus caillei*, Emasculation techniques, Heterostyly

Introduction

In West Africa two okra types exists, the early and the late okra types. The early okra which is synonymous with common or conventional okra, and found in all the okra growing regions of the world is *Abelmoschus esculentus* (L.) Moench, while late okra or West African or tall okra, on the other hand, which is restricted in distribution to the West African region, hence the name West African okra, is *Abelmoschus caillei* (A Chev.) Stevels The differences between these two cultivated okra types have been highlighted by Njoku(1958), Singh and Bhatnagar(1975), Oyolu(1977), Martin and Ruberte (1978), Nwoke (1980), Martin (1982), Siemonsma(1982), Udengwu (1988), Hamon and Hamon (1991), Hamon and van Sloten (1995), Siemonsma and Hamon (2004)

Incidentally, not much is known about late okra, since research in okra in the West African region, where it grows naturally, has been very scanty. Singh and Bhatnagar (1975) observed that West African okra contains many disease resistant genes which can be used to improve the conventional okra. To fully determine and exploit the potentials of this West African okra, its peculiar attributes must be fully studied and documented and compared with conventional okra.

In the course of carrying out hybridization studies with both okra types during the dry and rainy seasons, it was discovered that some okra types were very sensitive to the conventional emasculation technique in okra as was used by Purewal and Randhawa (1947), Joshi *et al* (1958) and Fatokun *et al* (1979), during the different seasons resulting in the loss of very important crosses. In their studies, Morakinyo and Adeyemi (2007) noted that the effect of seasons is evident in the magnitude of the variances in the eighteen characters they studied in okra. To effectively address this problem, alternative emasculation techniques would be necessary.

It was equally found that a certain degree of heterostyly existed within the cultivars used. Consequently, the Destructive Emasculation Technique (DET) and the Non- Destructive Emasculation Techniques (NDET) were developed. The two emasculation techniques were compared over the dry and rainy seasons to find out which one is better for okra breeding in the region, during each season. The DET is a modification of the technique used by Purewal and Randhawa (1947) while NDET is new to the author's best knowledge.

This present study reports on the comparative analysis of the response of the two okra types, to the two emasculation techniques, over the dry and wet seasons. It recommends NDET as a better emasculation technique in the West African region for both seasons. It also reports the existence and classification of heterostyly in okra and its implications in okra emasculation for more effective and rapid breeding studies.

Materials and Methods

Rainy season planting

Establishment of the experimental plants: Three early okra types; Awgu early, Kano dwarf and Obimo girdle and three late okra types; Ogolo, Oru ufie and Ebi ogwu were used for these studies. For the rainy season planting the six cultivars were grown in the Botanical Garden, University of Nigeria, Nsukka.

The plantings were done in early August on a flat bed measuring 2.5 m x 5.0m. Planting in early August was necessary to enable both okra types flower at the same time since the late okra can only flower around September when natural day length becomes less than its critical day length of 12¼ hrs (Njoku, 1958; Oyolu, 1977; Nwoke, 1980 and Udengwu, 1998). Early okra on the other hand can flower at any time of the year. Well cured poultry manure was applied to the beds at the rate of 1.6 metric tons per hectare, seven days before planting.

The six cultivars were randomly planted in rows, using a table of random numbers, with 30cm x 30cm bed spacing. Three pre-germinated seeds were planted per hole and this was later thinned down to one per stand when the plants were fully established. This gave a total of 12 plants per cultivar and 72 plants in the plot or 57600 plants per hectare. The leaf borer, *Podagrica uniforma* was controlled by weekly foliar application of Vetox-85, at the rate of .7kg/ha, using a Knapsack spray. The two emasculation techniques were used when the plants started flowering. Emasculation was limited to the first five flowers of each plant.

Emasculation techniques

Destructive emasculation technique (DET): As the name suggests, this technique which is a modification of the technique used by Purewal and Randhawar (1947) entailed the complete removal of the following parts of the flower: the calyx, the corolla, the anthers and filaments, using a small forceps. A flower that would open the following day was normally used and this was easily identified by the change of colour of the cone-shaped calyx from light green to light yellow.

Oftentimes towards the evening of the day preceding anthesis, a slit (S) can be seen on the tip or apex of the "calyx cone" which exposes part of the yellow corolla (Plate1).

Plate 1: Slit on a flower bud to open next morning

To carry out DET, a small forceps is used to extend the split to the base of the flower near the ovary. If the slit did not occur, the forceps was used to make a split down towards the base. Three more splits were made around the calyx to complete a North, South, East and West directional splits which resulted to a rather easy removal of the entire calyx from the base of the calyx (Plate 2). This is followed by careful removal of the five petals, from the base using the forceps (Plate 3). Thereafter the anthers and filaments were scrapped off from the staminal column leaving only the stigma lobes above the staminal column (Plate 4).This was covered with a cone-shaped paper clipped together at the base with a paper clip. Pollination was carried out the following morning at 8.00 hours, when the stigma had become receptive. The pollinated flower was left covered until the staminal column, stigma and style had dropped.

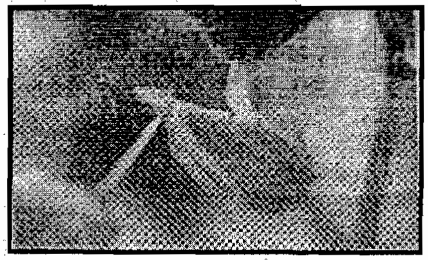

Plate 2: Making of four slits on the mature flower bud prior to removal of the calyx

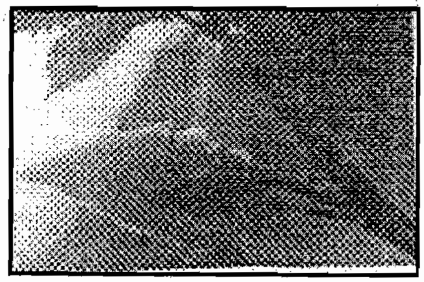

Plate 3: Removal of corolla after removal of calyx

Plate 4: Exposure of unreceptive stigma after removal of calyx and corolla

Non destructive emasculation technique (NDET): Unlike Destructive Emasculation Technique, the NDET puts into cognisance the type of heterostyly associated with the flower to be emasculated. In NDET, emasculation is carried out on a flower very early in the morning as the petals are about to open and before the yellow pollen grains are shed and insects begin to visit the flowers. In this technique, a flower that was about to open in a particular day was visited between 6.00 - 7.00 hours. With both hands the corolla that was about to open was forced open, care being taken to avoid breaking the petals. At this time the anthers are beginning to dehisce and the stigma slightly sticky. Once the petals were forced open, with the aid of the forceps, the stamen tube was removed in four strips together with the anthers and filaments. Whether the removal would start from the base or top of the stamen tube often depended on the type of heterostyly exhibited by the flower.

The three types of heterostyly identified were "Epistigmatic", "Peristigmatic" and the "Hypostigmatic" types. In the "Epitigmatic" type

(Plate 5), the upper part of the stamen tube bearing the anthers and filaments cover the surface of the stigma. For the "Peristigmatic" type (Plate 6), the tip of the upper part of the stamen tube is almost at the same level with the surface of the stigma. While for the "Hypostigmatic" type (Plate 7), the upper part of the stamen tube is clearly below the entire stigma.

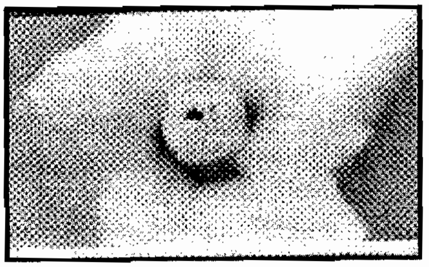

Plate 5: Epistigmatic condition in an okra flower

Plate 6: Peristimatic condition in an okra flower

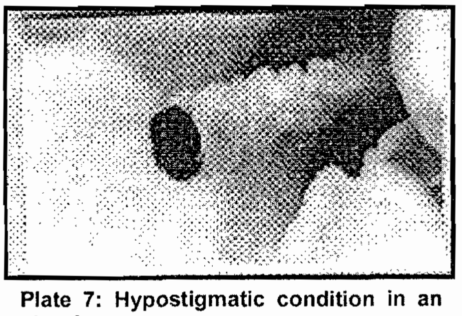

Plate 7: Hypostigmatic condition in an okra flower

If the heterostyly was of the "Epistigmatic" or "Peristimatic" types, four cuts were made at the base of the staminal column with a micro scissors. Caution was exercised to avoid damaging the ovary which is embedded in the swollen base above the receptacle. With the help of the forceps the four strips were pulled up from the base to the top. This is called the Down-Up approach. Once completed the style could be seen covered by the stamen tube (Plate 8). There was no fear of the pollen grains dusting on the stigma since at this time the anthers had only split without folding on their backs (an action necessary for the shedding of the pollen grains). However even if a stray pollen got deposited on the surface of the stigma it was easily spotted because of the sharp contrast between the yellow pollen grains and the maroon red stigma. In such a case the pollen was easily removed using a Carmel brush dipped in 70% alcohol. With the emasculation completed, pollen grains from the desired parent were dusted on the surface of the stigma. If the heterostyly was of the "Hypostigmatic" type then the removal of the stamen column began from the top of the column to the base (Up-Down approach) (Plate 9). A small scissors was used to cut off each of the strips thereby exposing the stamen tube covering the style as well as the upper part of the ovary (O), at the base of the flower and pollination effected. There is no need to cover the flower once the stigma lobes were completely dusted with pollen grains.

Plate 8: Down-up removal of anthers and filaments in four slits (DET)

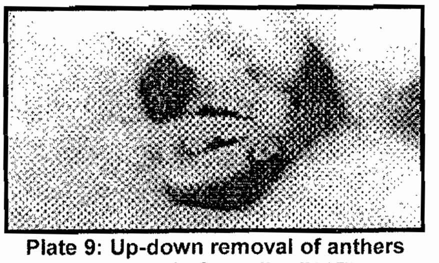

Plate 9: Up-down removal of anthers and filaments in four slits (NDET)

Dry season plantings

Establishment of the experimental plants: The establishment of the experimental plants for the dry season planting was similar to that of the rainy season planting. This was done towards the end of November in the Botanical garden, and since this was the dry season the plants were mulched with dry grasses from the field. Watering was done twice daily, morning and evening. When the dry season plants started flowering, emasculation and crosses were repeated as described in the rainy season planting and the results recorded for comparison with the results from the rainy season planting.

Results and Discussion

Table 1 gives a summary of both the total number of successful and unsuccessful crosses, involving the two okra types, for NDET and DET over the two seasons. The percentage number of successful crosses for DET (early) was 68% and 60% for rainy and dry seasons respectfully while for NDET (early) it was 79% and 75% for rainy season and dry season respectively. The comparison of DET(early) and NDET(early) using Contingency Chi square (χ^2) statistic (Table 2) shows that there was no significant difference (P>.05) in the response of early okra to the two treatments during the rainy season, but there was a significant difference

Table 1: Total Number and Percentage of Successful and Unsuccessful Crosses, using Early and Late Okra Types, During the Rainy and Dry Seasons

Emasculation Techniques	Total Number of Crosses		Number of Successful crosses		Percentage of successful crosses		Number of Unsuccessful crosses		Percentage of unsuccessful crosses	
	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season
DET -Early(E)	116	110	79	66	68%	60%	37	44	32%	40%
NDET (E)	92	102	73	77	79%	75%	19	26	21%	25%
DET-Late (L)	117	120	93	86	80%	72%	24	34	20%	28%
NDET (L)	124	115	116	103	94%	90%	8	12	6%	10%
DET (E&L)	233	230	172	152	74%	66%	61	78	26%	34%
NDET (E&L)	216	217	189	180	88%	83%	27	37	12%	17%

Table 2: Test for Significance in Relation to Emasculation Technique and Season of Planting, in Early and Late Okra, using Contingency Chi Square (X^2) Statistic

Source of comparison	Chi-square (χ^2) value		Degree of freedom		Level of significance	
	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season
DET(Early) - NDET(Early)	3.55	5.43	1	1	NS P>.05	* P<.05
DET(Late) - NDET(Late)	10.41	17.89	1	1	** P<.01	***P<.001
DET(Early) - DET(Late)	3.85	3.80	1	1	* P<.05	NS P>.05
NDET(Early) - NDET(Late)	3.72	10.30	1	1	** P<.01	**P<.01
DET(E&L) - NDET(E&L)	12.70	15.08	1	1	***P<.001	***P<.001
DET(Rs) - (DET(Ds)		3.31		1		NS
NDET(Rs) - NDET(Ds)		1.79		1		NS
DET-E(Rs) - DET-E(Ds)		1.91		1		NS
DET-L(Rs) - DET-L (Ds)		2.27		1		NS
NDET-E(Rs) - NDET-E (Ds)		0.45		1		NS
NDET-L(Rs) - NDET-L(Ds)		0.86		1		NS

DET-E= Destructive Emasculation Technique Early, DET-L= Destructive Emasculation Technique Late, NDET-E= Non-Destructive Emasculation Technique Early, NDET-L= Non-Destructive Emasculation Technique Late, Rs= Rainy season, Ds= Dry season

(P<.05) in its response during the dry season. Similarly, DET (late) recorded 80% success and NDET (late) 94% success for the rainy season. For the dry season the percentage success for DET (late) was 72% and 90% for NDET (late). Again when these performances were compared, using Contingency Chi square (χ^2) statistic (Table 2), the results showed that the response of late okra to the two treatments during the rainy season was highly significant (P<.01). For the dry season, the response was very highly significant (P<.001). The sources of these significant responses can be seen in, Table 1, where DET(early) recorded 60% success as against 75% success for NDET(early) during the dry season. DET (late) recorded 80% success as against the 94% for NDET (late) for the rainy season and 72% success for DET (late) as against 90% for NDET (late) during the dry season.

The combined responses of both okra types, for DET(E&L) on the one hand and for NDET(E&L) on the other hand, (Table 1), showed that percentage success for DET was 74% and 88% for NDET for the rainy season. For the dry season, the percentage success was 66% for DET and 83% for NDET. The comparisons of these responses DET(E&L) – NDET(E&L), Table 2, showed that there were very highly significant differences (P<.001) in the combined responses of the two okra types to the two treatments.

An obvious reason for the higher significant responses recorded for both NDET(early) and NDET(late) as well for the combined response of both okra types, NDET(E&L), is that since the technique only entails the removal of the staminal column with every other part of the flower intact, it inflicted by far less injury on the flowers. Additionally it had fewer exposed surfaces which could serve as entry points for disease causing organisms capable of aborting emasculated and pollinated flowers. Two other consequences of removal of almost all the non-essential parts of the flower in the DET treatment include the obvious shock the flower could receive from such an un-natural treatment. Such shock could be responsible for the abortion of the flowers. The other consequence is that due to the relatively large exposed surfaces of the flower, the rate of water loss could be much higher leading to dehydration and also possible abortion of the pollinated flowers especially during the dry season planting.

For NDET (early), Table 1, the percentage success was 79% for the rainy season and 75% for the dry season, while for NDET (late), the percentage success was 94% for the rainy season and 90% for the dry season. A comparison of the two treatments for the two seasons using Contingency chi square (χ^2) statistic, Table 2, showed that the differences in response between the two treatments were very significant (P<.01) for the two seasons. Undoubtedly late okra outperformed early okra for both seasons. This could be an indication of the more resilient nature of late okra which is believed to confer on it resistance to many diseases known to severely attack early okra, as has been highlighted by Singh and Bhatnagar(1975), Martin (1982), Siemonsma(1982), Udengwu (1988), Hamon and Hamon (1991), Hamon and van Sloten (1995), Siemonsma and

Hamon (2004). Table 1 also showed that the percentage success for DET (early) was 68% for rainy season and 60% for dry season. For DET(late), it was 80% for the rainy season and 72% for the dry season. A comparison of the results of the two treatments, over the two seasons (Table 2), shows that the rainy season treatment was not significant (P>.05), while the dry season treatment was significant (P<.05). This result suggests that though the destructive emasculation technique proved to be relatively harsh to the two okra types during the dry season, (the harshness being more pronounced in early okra), late okra was able to withstand this shock better than early okra. This better performance of, the long neglected late okra, which is indigenous to West Africa, may not be unconnected with its higher chromosome number (2n = 194) as against the (2n = 130) of early okra (Singh and Bhatnagar, 1976; and Udengwu, 1988). This might have provided it with more, and or better gene combinations resulting to a tougher nature and greater resistant to environmental changes, as well as a perennial tendency, among other attributes, already pointed out by Martin and Ruberte, 1978; Siemonsma ,1982; Martin,1982; Udengwu,1988 ;Hamon and Hamon, 1991; Hamon and van Slotan,1995; Siemonsma and Hamon, 2004 and Morakinyo and Adeyemi 2007.

From Table 1, though the percentage successes for the rainy season were higher than those of the dry season for all the treatments, the overall responses of both okra types to DET on one hand and NDET on the other hand for the two seasons were however not significant (P>.05) based on the comparison using Contingency chi square (χ^2) statistic (Table 2). Similarly a comparison between the responses of early okra alone to the DET and NDET treatments separately for the two seasons again showed that their responses were insignificant. The same situation also applies to the separate response of late okra to the DET and NDET treatments for the two seasons. These results are possible indicators to the point that success of emasculation in okra, in the area where the studies were carried out and possibly the West Africa region, depends not much on the season but rather on the type of technique employed.

A possible reason for this is that as Martin and Ruberte (1978) noted, okra is a well suited vegetable crop for the hot humid environments south of the savanna. The climatic changes occasioned by the shift from the rainy season to the dry season and vice versa may not be adverse enough to cause an appreciable difference in the response of both okra types to these emasculation techniques. This observation is however different from the results of Morakinyo and Adeyemi (2007), which showed that in both seasons the accessions of okra they analysed differed significantly with respect to all the eighteen characters they studied (P<.01). They further noted that the effect of the seasons was evident in the magnitude of the variances, particularly for final plant height, plant height at flowering, number of days to flowering and number of leaves per plant.

From this comparative analyses, one can say that NDET appears to be a better emasculation technique for both okra types and is ideal for both the sensitive as well as the non sensitive types. Its development locally underscores the need for researchers in the developing worlds, as Okonkwo (1984) noted, to carry out *in situ* research to find out methods and materials best suited for their environments rather than slavishly copying what had been developed for other parts of the world. The NDET is therefore recommended for breeding studies in okra and other related members of the Malvaceae family in the West African sub region.

Heterostyly in okra was briefly reported here because of its importance in the non- destructive emasculation technique. Obviously it is an issue that merits further screening and selection, for as yet no single plant was identified as showing only one type of heterostyly. It is however believed that with further studies, types could be isolated which may be more inclined to cross pollination, by making self pollination difficult if not impossible. Such identification will be a welcome development in okra breeding.

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