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Characterization and Cross-Compatibility in Interspecific Hybridization of Jute (*Corchorus spp.*)

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Abstract

Jute (*Corchorus*) species are widely distributed and cultivated for fibers, vegetables and traditional medicine throughout the tropics, subtropics and temperate regions. The objectives of this study were to (i) characterize the three *Corchorus* species (*Corchorus olitorius*, *Corchorus* incisifolius, and *Corchorus tridens*); and (ii) investigate the cross-compatibility of interspecific hybridizations within the genus *Corchorus.* The three *Corchorus* species were evaluated at botanical garden of University of Ilorin in 2016 and were crossed in all possible combinations using hand emasculation procedures to generate F_1 hybrids. Analysis of variance (ANOVA) was performed on the agronomic data collected on them. The difference between means was separated by the least significant difference (LSD). The percentage of successful and aborted crosses was calculated. The results obtained from the analysis revealed that *Corchorus incisifolious* shared common characteristics (monomorphic) with *Corchorus olitorius* such as yellowish leaf-lamina, glabrous hairiness, and medium seed-size, whereas *Corchorus tridens* had wine coloration in its leaf-lamina, pubescent hairiness of leaf, and small seed-size. Thus, the three species differed in some qualitative characters (dimorphic or polymorphic), such as leaf shape, seed coat color, and leaf apex, which were proposed diagnostic features. *Corchorus incisifolious* had the most leaves, longest pod, and widest pod based on quantitative characters. About 121 crosses were attempted between the three species of *Corchorus* using hand emasculation method, but only one cross combination was successful between *Corchorus olitorius* and *Corchorus incisifolious* (4.76%) out of 21 attempts, while other cross combinations failed. Therefore, qualitative characters are good genetic markers to distinguish cultivated *Corchorus* species, while sexual barrier and other factors were suggested to be responsible for the low success rate recorded in this study. Hence, biotechnology techniques (*in vitro* pollination or embryo rescue) can be used to overcome these barriers.

Introduction

Corchorus spp. is in the *Corchorus* genus, *Malvaceae* family and subfamily *Grewioideae* and is commonly known as jute (Basu *et al*., 2016). There are several species within the *Corchorus* genus and some of the most economically significant ones include: *Corchorus olitorius* **(**Nalta Jute or Tossa Jute**)**, *Corchorus incisifolious* (Native Jute or Nigerian Jute**),** and *Corchorus tridens* **(**Wild Jute or Bengal Hemp) (Ogunkanmi *et al*., 2010). *Corchorus* species are widely distributed throughout the tropics, subtropics, and warm temperate regions of the world (Bayer and Kubitzki, 2003). Although the origin and phylogeny of the *Corchorus* genus still remain controversial (Benor *et al.,* 2010), there is little information about the genetic and evolutionary relationship between wild *Corchorus* spp. and the cultivated species (Basu *et al.,* 2004; Roy *et al.,* 2006). However, the presence of more wild types of *Corchorus* species in Africa pointed to Africa as the center of origin of the genus, with a secondary center of diversity in the Indo-Burmese region (Makinde *et al*., 2009).

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The various species of the crop are typically woody herbs that grow to a height of about 0.75-2.00 m. They are glabrous and occasionally pubescent, especially those that are cultivated. *Corchorus olitorius* is one *Corchorus* species that is raised for its long, strong jute fibers, which is used in textiles, ropes, and sacks. In some cuisines, it is also used as a leafy vegetable. *Corchorus tridens* is cultivated for its traditional medicine, crafts, or as a wild plant resource whereas *Corchorus incisifolious* is occasionally grown as a fiber crop and is used in traditional medicine (Sinha *et al*., 2004; Khan, 2008; Anwar, 2011). According to reports, *Corchorus* species may improve male sexual prowess. This conclusion is based on personal experience and a synthesis of data from the traditionalists or herbalists who regularly consume these plants (Bhatt *et al*., 2003). The vitamins A and C in the leaves are abundant. Additionally, gonorrhea, chronic cystitis, and dysuria are among the illnesses that are treated with its leaves (Ogunkanmi *et al*., 2010).

Previous improvements of jute have been restricted to selection from natural population with quantitative traits like height and fiber quality as the target characters. As a result, *Corchorus* species had narrow genetic diversity (Saha *et al*., 2001). Interspecific hybridization of wild and domesticated species may therefore result in the preservation of wild germplasms and the development of a wide genetic diversity in jute cultivars (Roy *et al*., 2006; Maity *et al*., 2012). Interspecific hybridization becomes essential and has been used to expand the genetic base and develop new cultivars by transferring desirable traits (Sinha *et al*., 2004). New cultivars with stable, and excellent characteristics can be developed quickly using various hybrid combinations (Xing *et al*., 2022). This method has been used successfully in eggplant, loquat, azalea, tulip, and some other important crops (Devi *et al*., 2015; Li *et al*., 2016; Xie *et al*., 2019; Xing *et al*., 2020). There is a dearth of information in Nigeria on the genetic improvement of jute through interspecific hybridization. The goal of this study was, therefore, to characterize the three *Corchorus* species (*Corchorus olitorius*, *Corchorus incisifolius*, and *Corchorus tridens*) and investigate the cross-compatibility of interspecific hybridizations within the genus *Corchorus* using interspecific hybridization. The information would aid the development of a comprehensive breeding strategy and broaden genetic diversity, which would aid improved seed and fiber production of the crop to meet the demand of jute farmers, textile industries, and other stakeholders in Nigeria.

Materials and methods

Genetic materials and experimental location

Seeds of three species of *Corchorus* (*C. tridens*, *C. incisifolious* and *C. olitorius*) were obtained from the botanical garden of University of Ilorin, Ilorin, Kwara State, Nigeria. The research work was carried out at the botanical garden of the University in 2016, which lies between latitude 8º30´N and longitude 4º33´E and the location has a tropical savanna climate (Köppen climate classification Aw).

Phenotypic evaluation of the three species of *Corchorus* for agronomic characters

Five polythene pots were used per specie with three replications, making 15 polythene pots per species and 45 polythene pots for the three species, while holes were made around to allow drainage and filled with rich sub-soil. The soil was wet to prevent dryness in readiness for planting. The dormancy of the seeds was broken by putting the seeds in a white handkerchief, tied, held properly, and then immersed in hot water for five seconds. This was done five times to break the seed dormancy (Schippers *et al*., 2002; Fondio and Grubben, 2011). The experiment was laid out in a randomized complete block design (RCBD). Two seeds were sown the following day in the previously prepared pots at the botanical garden of University of Ilorin. Watering was carried out daily in the morning and evening till maturity. Seedlings were thinned to one, after two weeks of planting (2 WAP) and the pots were mulched to reduce the rate of evaporation.

Interspecific hybridization among three species of *Corchorus spp.*

Hybridization was carried out using hand emasculation procedures (TNAU, 2023) in the morning and evening. The differences in the time of pollination was because the time the flowers of the three species open differs. The flowers of *C. olitorius* and *C. incisifolious* open early in the morning while those of *C. tridens* opens in the evening after sunset. Hence, by the time the *tridens* flowers start opening, *olitorius* and *incisifolious* are already closing. The buds to be used were emasculated a day before

anthesis, and all buds were removed from the spike. The buds were carefully opened with forceps, and the anthers were removed using forceps. Tissue paper was then used to bag the emasculated buds to prevent contamination by unwanted pollen and easy identification of the buds to be pollinated. Pollens from opened flowers of a species were collected and dusted on the stigma of the already emasculated buds of another species (the emasculated plant serves as the female parent, while the species from which the pollen is coming serves as the male parent), while tagging was done with the aid of thread, pencil, and a plastic tag.

Data collection

Plant height was the average of ten randomly selected plants measured with a transparent ruler and taken in centimeters at first flowering from ground level to the tip of the plant. Days to 50% flowering was taken when 50% of plants have begun to flower. Number of leaves per plant was determined by counting the number of leaves taken from ten plants at random and calculating the mean. Leaf length and width were also determined when the average of ten randomly selected plants were measured at flowering using a transparent ruler, taken in centimeters and recorded. Number of pods/plant was calculated as the mean of five representative plants were calculated and recorded. Number of buds/plant was calculated by counting ten randomly selected representative plants and the results were averaged. Pod and seed weights per plant were calculated by dividing total pods and seeds weights by number of plants at harvest and measured in gramme, on the other hand, qualitative characters such as laminal leaf pigmentation, stipule pigmentation; stem pigmentation; leaf shape; seed coat colour; seed size; hairiness of leaf; leaf pigmentation; leaf margin; nature of leaf base and nature of leaf apex were also collected based on Loumerem and Alercia (2016) procedures.

Data analysis

Analysis of variance (ANOVA) was performed on the agronomic data collected using Statistical Tool for Agricultural Research (STAR, version 2.0.1) software. The difference between means was separated by the least significant difference (LSD) at 5% and 1% levels of significance. For hybridization between three species of *Corchorus*, the percentage of successful and aborted crosses was calculated as follows:

$$\text{Percentage successful crosses (\%)} = \frac{\text{Number of successful crosses}}{\text{Number of crosses attempted}}\ x\ 100$$

$$\text{Percentage aborted crosses (\%)} = \frac{\text{Number of aborted crossess}}{\text{Number of crosses attempted}}\ x\ 100$$

Results and Discussion

Characterization of three species of *Corchorus* based on qualitative characters

The results obtained revealed that both *Corchorus olitorius* and *Corchorus incisifolious* had some qualitative characters (monomorphic) in common, such as yellowish leaf lamina with green coloration in leaf petiole, stipule, leaf, and stem. Both also had medium seed size and glabrous hairiness of leaf. On the other hand, *Corchorus tridens* exhibited wine coloration in its leaf lamina with green coloration in leaf petiole and stem with greenish leaf, serrated leaf margin. Similarly, *Corchorus tridens* had small seed size, and pubescent hairiness of leaf. In addition, the three *Corchorus* species had variations in some qualitative characters (dimorphic or polymorphic) such as leaf shape, for instance, *Corchorus olitorius* had lanceolate and ovate, *Corchorus incisifolious* exhibited ovate lobed, whereas *Corchorus tridens* had lanceolate. They also had varied seed coat color such as chocolate and brown in *Corchorus olitorius*, green and brown in *Corchorus incisifolious*, while *Corchorus tridens* had chocolate with wine color. Based on the nature of leaf apex, both *Corchorus incisifolious* and *Corchorus tridens* had acuminate, while *Corchorus olitorius* exhibited acuminate, mucronate leaf apex (Table 1, Fig. 1). Thus, description of *Corchorus incisifolious* leaves conforms to the findings of Ogunkanmi *et al*. (2010), while that of *C. olitorius* and *C. tridens* leaves is in conformity with the report of Osawaru (2012). The serration on the margin and the setae on the leaf base may be diagnostic features of Genus *Corchorus*. The color of the plant, serration, and presence of setae are suggested diagnostic features of *Corchorus* (Osawaru, 2012). Leaf margin is also one of the most important factors in distinguishing cultivated *Corchorus* species as observed in this study. This assertion is in line with the findings of Osawaru *et al*. (2012) who reported that foliar characters are more

Table 1: Qualitative traits of three species of *Corchorus* evaluated at botanical garden of the University of Ilorin in 2016

	Corchorus species		
Characters	*Corchorus olitorius*	*Corchorus incisifolious*	*Corchorus tridens*
Laminal leaf pigmentation	Yellow	Yellow	Wine
Leaf petiole pigmentation	Green	Green	Wine
Stipule pigmentation	Green	Green	Winish green
Stem pigmentation	Green	Green	Wine
Leaf shape	Lanceolate, ovate	Ovate lobed	Lanceolate
Seed coat colour	Chocolate and brown	Green and brown	Chocolate with wine colour
Seed size	Medium	Medium	Small
Hairiness of leaf	Glabrous	Glabrous	Pubescent
Leaf pigmentation	Light green	Dark green	Green
Leaf margin	Highly serrated	Deeply serrated	Serrated
Nature of leaf base	Acute, setae, round	Round, setae	Acute, setae
Nature of leaf apex	Acuminate, mucronate	Acuminate	Acuminate

Fig. 1: Pictorial overview of the qualitative characters of three species of *Corchorus*

important in characterizing *Corchorus* species. The use of foliar characters in distinguishing cultivated *Corchorus* species may be due to the fact that many mutants have not yet been accumulated in the jute population due to a lack of human selection pressure for a longer period of time (domesticated only around 200 years ago) (Mukherjee and Kumar, 2002). The findings of this study revealed that most of the qualitative characters were monomorphic with few dimorphic characters. This agrees with the report of Das and Kumar (2014), who reported that the qualitative characters of jute are mostly monomorphic and a few are dimorphic and polymorphic.

Characterization of three species of *Corchorus* based on quantitative characters

The results obtained (Table 2) showed that *Corchorus tridens* reached 50% flowering earlier (47 days after planting), whereas *Corchorus incisifolious* flowered later (70 DAP) than the rest. The number of leaves per plant differed significantly, as *C. incisifolious* had the highest number of leaves (83) while *C. tridens* had the lowest (59). The number of leaf per plant has been reported to be of great importance to vegetable growers and breeders (Adebo *et al.,* 2015). The pod length was also significantly different among the species, with *C. incisifolious* being the longest (6.32 cm), while the shortest was recorded in *C. tridens* (4.60 cm). In addition, the pod of *C. incisifolious* was the widest (3.74 cm), while the narrowest pod was obtained from *C. tridens* (0.52 cm). However, *C. tridens* had the highest number of pods per plant (48) with the highest number of buds (17), whereas *C. incisifolious* had the least. In terms of pods and seed weights per plant, *C. incisifolious* had the highest with mean values of 0.66 g and 0.47 g per plant, respectively, whereas *C. tridens* had the lowest values (0.23 g and 0.13 g respectively). It could be deduced that *C. incisifolius* showed superiority in most of the quantitative characters measured in this study. This could due to its inherent genetic potential, long-term domestication, and adaptation in Nigeria, as earlier reported by Ogunkanmi *et al*. (2010).

Interspecific hybridization among three species of *Corchorus spp.*

The results of the number of attempted crosses and the percentage of successful crosses among the three species of Corchorus are presented in Table 3. One of the primary techniques for developing new plant cultivars is interspecies or inter-generic hybridization (Nishio et al., 2019).

Table 2: Mean values for agronomic characters of the three species of *Corchorus* evaluated at botanical garden of the University of Ilorin in 2016

Species	Days to 50% flowering	Plant height (cm)	Number of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Pod length (cm)	Pod width (cm)	Number of pods/plant	Petiole length (cm)	Number of buds/plant	Pod weight (g)	Seed weight (g)
Corchorus tridens	47.00± 0.00^b	83.84 ± 6.72^b	59.40 = 7.92^b	8.30 ± 0.55^a	1.92 ± 0.46^b	4.60 ± 0.59^b	0.52 ± 0.13^c	48.20± 6.14^a	1.32 ± 0.41^a	17.00 = 3.81^a	0.23 ± 0.03^b	0.13 ± 0.03^b
Corchorus Olitorius	49.00± 0.00^b	92.44 ± 18.66^b	64.20 = 5.21^b	6.86 ± 1.76^b	2.24 ± 0.35^a	4.98 ± 0.82^b	2.08 = 0.80^b	15.20 ± 5.31^b	1.74 ± 0.85^a	13.60 = 4.39^b	0.40 ± 0.07^a	0.31 ± 0.08^a
Corchorus incisifolious	70.00 ± 0.01^a	113.76 ± 16.12^a	82.60 = 13.84^a	5.40 ± 0.85^b	3.78 ± 0.40^a	6.32 ± 0.55^a	3.74 ± 0.39^a	19.20 ± 10.06^b	1.80 ± 0.43^a	6.00 ± 2.00^c	0.66 ± 0.10^a	0.47 ± 0.10^a
F-test	*	*	*	**	*	*	*	*	Ns	*	*	*

*, ** Significant at ($p < 0.05$) and ($p < 0.01$) respectively; Means with the same letter(s) in the same column are not significantly different from each other at p = 0.05; ns = not significant at 0.05

Table 3: Crosses made among the three species of *Corchorus* (*tridens, incisifolious* and *olitorius)* at botanical garden of the University of Ilorin in 2016

Possible combinations	Number of crosses attempted	Number of successful crosses	Number of aborted crosses	Percentage successful crosses (%)	Percentage aborted crosses (%)
tridens × *olitorius*	21	Nil	21	0	100
tridens × *incisifolious*	19	Nil	19	0	100
olitorius × *tridens*	21	Nil	21	0	100
olitorius* × *incisifolious	21	1	20	4.76	95.24
Incisifolious × *tridens*	24	Nil	24	0	100
incisifolious × *olitorius*	15	Nil	15	0	100

A total of 121 crosses were attempted between the three species of Corchorus using the hand emasculation method. Only one cross combination was successful between Corchorus olitorius and Corchorus incisifolious (4.76%) out of 21 attempts, while the other cross combinations failed. Parent fertility is a key factor influencing the seed-setting rate of interspecific hybridization (Anwar, 2011; Alexander, 2019). The low success rate (4.76%) between Corchorus olitorius and Corchorus incisifolious, with no successful crosses in other combinations, suggests the presence of pre-zygotic and post-zygotic compatibility barriers among the three Corchorus species used. Carrera et al. (2009) observed pod drop after pollination in some crosses, indicating abnormal embryo development and incompatibility at pre-zygotic and post-zygotic stages. The pre-zygotic barrier, also known as the pre-fertilization barrier, may be due to pollen tubes becoming stuck in the female stylar canals (Marasek-Ciolakowska et al., 2018). Even with successful fertilization, post-zygotic barriers hindered embryo development and seed formation. Marasek-Ciolakowska et al. (2018) suggested that post-zygotic barriers could result from the degeneration of hybrid embryos and/or endosperm in incompatible crosses. Biotechnology techniques such as in vitro pollination or embryo rescue could potentially overcome these barriers for successful interspecific hybridizations in Corchorus. Similar barriers have been overcome in other crops using biotechnology techniques (Marasek-Ciolakowska et al., 2018; Okazaki, 2005).

Environmental conditions at the botanical garden during hybridization

Botanical gardens offer valuable settings for carrying out hybridization experiments where screen houses are not available for other crops. However, the success of the hybridization depends on critical weather conditions such as temperature, relative humidity, rainfall or irrigation, photoperiod sensitivity, pest control and disease control. Information on the relative humidity and temperature during the hybridization exercise between February and June, 2016 is presented in Table 4. In this study, the climatic conditions at the botanical garden revealed that relative humidity ranged from 40.66% (February) to 73.4% (June), with temperatures ranging from 27.0 ^{0}C (June) to 30.9 ^{0}C (March). Relative humidity and temperature above 27 ^{0}C (room temperature) could have impacted on the success of crosses and seed development in this study. Several researchers (Singh *et al*., 2019; Amusa *et al*., 2021) had reported that weather conditions such as temperature and relative humidity have a significant impact on hybridization in jute plants, affecting flowering, pollination, seed development, seedling establishment, and timing.

Table 4: Relative humidity and temperature during the hybridization exercise at botanical garden of the University of Ilorin in 2016

Months	Relative Humidity (%)	Temperature (^{o}C)
February	40.66	30.1
March	54.86	30.9
April	59.05	30.3
May	67.32	28.8
June	73.40	27.0

Conclusion

The results obtained from this study revealed that three *Corchorus* species differed in some qualitative characteristics (dimorphic or polymorphic), such as leaf shape, seed coat color, and leaf apex nature, which were proposed diagnostic features. *Corchorus incisifolious* had the most leaves, the longest pods, and the widest pods. About 121 crosses were attempted between three species of *Corchorus* using hand emasculation procedure, but only one cross combination was successful between *Corchorus olitorius* and *Corchorus incisifolious* (4.76%) out of 21 attempts, while other cross combinations failed. Therefore, qualitative characters are good genetic markers to distinguish cultivated *Corchorus* species, while sexual barrier and other factors were suggested responsible for the low success rate recorded in this study. Hence, biotechnology techniques (*in vitro* pollination or embryo rescue) can be used to overcome these barriers.

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