Original Research Article

IN PLANTA ANDROGENESIS (PATERNAL ORIGIN) IN *SAACHARUM* X *ERIANTHUS* INTERGENERIC HYBRID

ABSTRACT

The present investigation was carried in crosses made between *Saccharumspecies* hybrid clones i.e. Co 419, Co 7201 and Co 7224 (2n = 108-112) and *Erianthusarundinaceus*viz. IK 76-91 (2n = 60) and IK 76-99 (2n = 60) for introducing better ratoon ability, vigour, wide ecological adaptability, extensive root system, drought and water logging resistance, high biomass and disease resistance into modern sugarcane cultivars during 2020-23at VSI-Sugarcane Breeding Centre, Amboli. Parental apomixis (paternal origin) has been recently noticed and it is unique in *Saccharum* X *Erianthus*intergeneric cross seedlings. A total of fifty-one seedlings were obtained from above three intergeneric crosses and out of these eighteen seedlings were reported as androgenetic seedlings as the female act as a surrogate mother. At the early stages, seed germination of seedlings was found two types i.e., one type resembling *Saccharum* and other resembling *Erianthus* with deep purple coloration. Morphologically androgenetic seedlings were similar to the male parents IK 76-91 and IK 76-99 with tall large tufted grass like clumps, vegetative stem with thick reproductive buds, root zone narrow and only one row of root eyes, the lamina gradually passes into leaf sheath with no auricle and dewlaps. Cytological studies were made in these seedlings and during metaphase the somatic chromosome number was observed to be 2n = 60 rather than expected 2n = 84-90.This paternal origin may be due to the ability of *Erianthus* male gametes to produce an embryo within the seed tissues of *Saccharumspecies* hybrids but without genetic contribution of the seed. Thus, *in planta* androgenesis is achieved through the combination of embryogenic behavior of male gametes which has entered in the ovule and the ability of ovules to act as a surrogate mother. They could derive either from the fusion of two male gametes or from the early natural diplodization of the haploid embryo. Such homozygous true breeding seedlings of *Saccharum* X *Erianthus* with hybrid vigour are extremely important in crop improvement. This system provides an unparalleled opportunity to shorten the breeding cycle and fix agronomic traits in the homozygous state. These androgenetic plants with *Saccharum* cytoplasm and *Erianthus* genome are male sterile making them highly useful for further hybridization and sugarcane improvement programme.

Key words: Androgenesis,cytology,*Erianthus*,intergeneric hybrid and*Saccharum*

Introduction:

Modern sugarcane cultivars are complex interspecific hybrids (2n = 100–130). They were developed from interspecific crosses made early this century among relatively few clones of *Saccharum officinarum* (2n = 80), the sugar producing species and *S. spontaneum* L. (2n = 40-128) a wild species. Breeders are concerned by the potentially narrow genetic base of contemporary commercial crops. The genera *Saccharum* L., *Erianthus*Mychx. *Section Ripidium*Henrad, *Miscanthus*Anderss, *Sclerostachya* (Hack.) *A. Camus* and *Narenga* Bor., which are closely related, where considered to be part of 'Saccharum complex' (Mukherjee 1957; Daniels *et.al* 1975) sharing a common gene pool.

Erianthusarundinaceus(Retz.) Jeswiet (2n = 60) belongs to the genus *Erianthus sect. Ripidium* is included in 'Saccharum complex'. It is a large grass with tall and thick stalk resembling sugarcane and has a great potential as a contributor of germplasm to current cultivars for better ratoon ability, vigour, wide ecological

adaptability, extensive root system, drought and water logging resistance, high biomass and disease resistance (Roach 1989 and D'Hont*et.al*.1995)

The genetic base of sugarcane is considered to be very narrow and gene introgression studies are being done to transfer the genes from wild species or related genera. Numerous attempts have been made to cross *E. arundinaceus* with sugarcane to introduce these characters into modern cultivars (Hale et al., 2022: Samantaray et al., 2020). However, no conclusive results have been achieved to date. Indeed, in only a few cases have conclusive evidences of true intergeneric hybrids being documented (Nagai *et.al* 1991, D'Hont*et.al*.1995, Besse *et.al*.1997a). One of the major obstacles in the past has been the identification of true hybrids using morphological characters.

Intergeneric crosses involving *Saccharum* spp hybrid as a female were made with *E. arundinaceus* as a male at Distant Hybridization Garden, Agali (Kerala), India in 2008 and morphological and cytological studies were taken up in the fifty-one seedlings obtained from the *Saccharum* X *Erianthus* three different crosses (ICAR). Hence, this study was undertaken for to shorten the breeding cycle by developing male sterile lines and fix agronomic traits in the homozygous state.

Materials and Methods

The *Saccharum spp.* hybrid Co 419, Co 7201 and Co 7224 were pollinated with *E. arundinaceus* (Retz.) Jeswiet clone IK 76-91 and IK 76-99 and the seedlings raised were maintained in the field during 2021-23 at VSI-Sugarcane Breeding Centre, Amboli. The somatic chromosome of the parental clones and the seedlings were determined by root tip squash technique. Actively growing root tips from cane cutting planted in pots with river sand were excited and treated in a saturated solution of α- bromonapthalene for about two hours. After thoroughly washing in distilled water the root tips were fixed in Ostergren and Heneen's (1962) fixative for about 24 hours. The root tips were hydrolyzed in 1N HCL for 15 minutes at 58 $\pm$ 2 ^{0}C and stained in Leuco basic fuchsin for 30 minutes and squashed in 1% acetocarmine. Well spread mitotic chromosomes were counted and photographed. The pollen fertility of the parents and the seedlings were tested with the help of Differentials stain (Alexander, 1969). The red stained pollen is categorised as the fertile pollen and unstained pollen as sterile.

Results and Discussion

Nine seedlings were obtained from *Saccharum* species hybrid; Co 7224 florets pollinated with *E. arundinaceus* clone IK 76-99 pollen, whereas six seedlings from the Co 419 X IK 76-91 and thirty-six seedlings from the Co 7201 X IK 76-91, respectively. All the seedlings in general were fast growing with good vigour. Among these fifty-one seedlings from the three crosses, eighteen seedlings were found to be androgenetic seedlings (Co 419 X IK 76-91: 06; Co 7224 X IK 76-99: 08 and Co 7201 X IK 76-91: 04).Morphologically these androgenetic seedlings were similar to the male parents IK 76-91 and IK 76-99 with tall large tufted grass like clumps, vegetative stem with thick reproductive buds, root zone narrow and only one row of root eyes, the lamina gradually passes into leaf sheath with no auricle and dewlaps. The chromosome number of *Saccharum* species hybrid Co 419, Co 7201 and Co 7224 and *E. arundinaceus* clone IK 76-99 weredetermined to be2n = 108-112 and 2n = 60 respectively. Intergeneric hybrid between these had expected n + n transmission with 2n = 84-90. During cytological study of seedlings about eighteen seedlings from the three different intergeneric crosses, the somatic chromosome number at metaphase recorded with 2n=60 (Fig.1b) and all the other seedlings were n + n hybrids with chromosome number with 2n= 84 to 90.

These eighteen intergeneric cross seedlings were morphologically and cytologically identical with the *Erianthus* male parent and recorded as androgenetic plant. No hairs on the eye bud (Fig. 1g, 1j), only one row of the root eyes (Fig.1h, 1k) and ligules are reduced (Fig. 1i, 1l) were present in all the androgenetic seedlings like male parents IK 76-91 and IK 76-99 (Fig. 1n, 1o). Two instances were recorded in the androgenetic seedlings, it had the similarity in morphologically and cytologically to the donor male parents. In looking to the morphological features of the *Saccharum* X *Erianthus* hybrid seedlings, the expected cytoplasm from *Saccharum* was missing with an element of uncertainty and it could derive either from the fusion of two male gametes or from the early natural diploidization of the haploid embryo. The pollen fertility was also studied in the parent and hybrid seedlings. The androgenetic seedlings (Fig. 1n, 1o) although had morphologically and cytologically similar to male parent but these seedlings had recorded with 100% sterile i.e. unstained pollen (Fig.1p).The female parent Co 7224 recorded with 28% fertility and showed both the red and unstained pollen (Fig.1q) and the male parents IK 76-91 and IK 76-99 were 100% pollen fertile with complete red pollen (Fig.1r). Until recently, apomixis had been reported only in Angiosperms. The highly endangered Mediterranean conifer, *Cupressus dupreziana* A. Camus (or Tassil cypress), is to our knowledge the first plant species where in planta "parental apomixis" (i.e. embryogenic development of diploid pollen in seed tissues) was hypothesized (Pichot *et.al*. 2000)

Sexual reproduction is the general rule in higher plants and animals. More than 99% of multicellular eukaryotes reproduce sexually (Bell 1982**;** Barton and Charlesworth 1998;West *et.al*1999). The sexual cycle follows the alteration of haploid and diploid cells delimited by meiosis and syngamy, two events that modify the nuclear chromosome number (Vrijenhoek 1998). However, in some asexual species in 40 angiosperm families (mainly Poaceae, Asteraceae, Rosaceae and Rutaceae) exhibit pattern of apomixis where the embryo is derived solely from maternal tissues, either somatic cell or a gamete with the somatic chromosome number (unreduced gamete) (Vielle Calzada *et.al*. 1996). In rare cases, the parthenogenetic development of a reduced gametophyte prodeces a haploid embryo (Foroughi-Wehr and Wenzel 1993).This last feature, also called non-recurrent apomixis, occurs naturally in some female gametophytes. It is also intensively used in breeding programs through the in vitro culture of male gametophytes (androgenesis). Diploid homozygous genotypes can then be obtained through or artificial doubling of the chromosome number.

The *E. arundinaceus*clones IK 76-91 and IK 76-99were collected from Kalimantan province in Indonesia and is with chromosome number 2n = 60 as that in other clones of this species collected from this region. They are hexaploids with basic chromosome number X =10 (Besse and McIntyre 1998). Sreenivasan (1987) reported that *Saccharum* X *Erianthus* hybridization looked most promising for transferring many desirable characters to present day commercial varieties, since these hybrids do not show significant reduction in sucrose content especially when *E arundinaceus* is used as a male parent. In the present investigation, the intergeneric hybrid seedlings recorded with 2n = 60 chromosome number which was not expected and it was to be 2n = 84-90 with normal n + n chromosome transmission. These hybrids were identical to the male parent IK 96-91 and IK 76-99 not only in respect of chromosome constitution but also in morphological features. Generally, in the intergeneric hybrid between *Saccharum* X *Erianthus*, the contribution of cytoplasm could be of *Saccharum* and the nuclear genome from both the genera chromosome contribution. In this study, eighteen hybrid seedlings from three different crosses, the *Saccharum* cytoplasm was eliminated by the *Erianthus* genera and occupied both the cytoplasm and nuclear genome and recorded as androgenetic plant. When *Erianthus* was used as a male parent

in hybridization with *S. officinarum* and Co 7201, two instances of androgenesis were observed. The seedlings had the morphology and chromosome number of the male parent (Anon, 1989-90).

The peculiarity that is almost unique in sugarcane hybridization is that no prediction can be made as to what chromosome number is a particular cross would be like. Many introgression programmes have been attempted without to success to introduce characters from *E. arundinaceus* to *Saccharum species* hybrids. Three major difficulties occurred at different steps of these intergeneric crosses programme (Piperidis*et al.*2000). The first difficulty is the production of progeny from intergeneric crosses with *E. arundinaceus*. Lee (1995) showed that incongruity between the pollen and pistils of the two genera occurs since he observed that the *Erianthus* pollen tube often did not reach the micropyle of the *Saccharum* ovules. They either stopped growing toward it or they lost directional control. This uncoordinated pollination process happens between distinct species (Hogenboom 1975) and resulted in limited intergeneric fecundation. In addition to sugarcane flowers are hermaphroditic with varying degrees of male sterility clones with less than 30% viable pollens are used as females in crossing. Thus because of incongruity between the pollen and pistils, even if the *Erianthus*parent is a strong male and female parent has little fertile pollen, the few crosses that produce seed generate mostly self-pollinated individual. The second difficulty was the identification of genuine hybrids using morphological characters, which has been highlighted by use of molecular diagnostic tools (D'Hont*et. al* 1995). The third difficulty is to maintain the genuine hybrids, which often are very week.

Using cytological tools, we were able to analyse chromosome number of eighteen intergeneric hybrid seedlings with 2n = 60 which were identical to the male *Erianthus*parent IK 76-91 and IK 76-99 and morphologically these plants were also similar in all respect with male parent. The cytoplasm was expected from *Saccharum* genera as it was used as a female parent. This paternal origin may be due to the ability of *Erianthus* pollen to produce an embryo within the seed tissues of *Saccharum* species but without genetic contribution of the seed, as reported Pichot *et al.* 2001 when *Cupressus sempervirens* seed trees were pollinated by *C. dupreziana* pollen. The androgenetic process occurred from unreduced diploid pollen of *C. dupreziana*and all the progeny from *C. sempervirens* seed trees were diploid. They could derive either from the fusion of genetically identical two male gametes (i.e. deriving from the same pollen) or from the early natural diploidization of the haploid embryo. Although the production of all maternal progeny by apomixis or parthenogenesis is a rather frequent phenomenon, the production of all paternal progeny has been very rarely reported (McKone and Halpern 2003). To our knowledge, *C. dupreziana* is the only plant in which progeny are produced by the apomictic development of pollen grains. The scarcity of androgenetic cases reported compared to the amount of gynogenic case reports may be explained not only by the probable evolutionary dead end of this reproductive process but also by the difficulty in detecting it (McKone and Halpern 2003). The male component ability to produce an embryo without female gamete contribution may not be so rare. In fact, it is intensively used in Angiosperms to produce haploid genotypes from in vitro culture of immature anthers. Thus, favourable growth conditions combined with a lack of syngamy opportunity may often lead to androgenesis. This paternal origin may be due to the ability of *Erianthus* male gametes to produce an embryo within the seed tissues of *Saccharumspecies* hybrids but without genetic contribution of the seed.

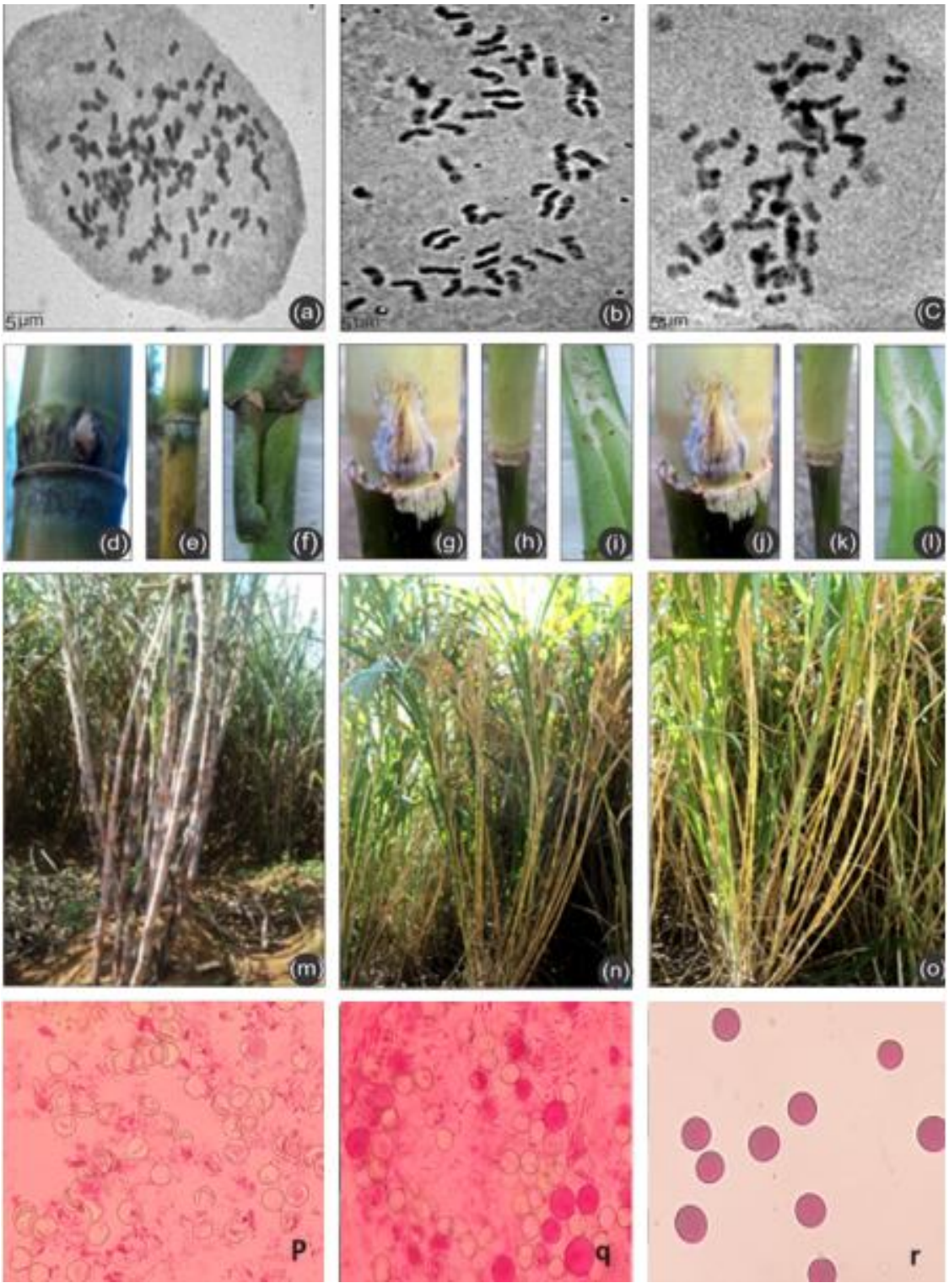

Fig 1. Somatic chromosome number at prophase, metaphase and anaphase (Fig. 1a, 1b and 1c),No hairs on eye bud (Fig. 1g, 1j), only one row of the root eyes (Fig. 1h, 1i) and reduced ligules(Fig. 1i, 1l), Androgenetic seedlings (Fig. 1n, 1o), unstained pollen grains (Fig. 1p) found in androgenic seedlings, stained & unstained pollen grains (Fig. 1q) found in female clones, and fully stained pollen grains (Fig. 1r) found in male clones, morphological traits hair on eye bud (Fig. 1d), 2-3 rows of root eyes (Fig. 1e) and hairs on ligules (Fig. 1f).

Conclusion

It is concluded that *in planta* androgenesis is achieved through the combination of embryogenic behaviour of male gametes which has entered in the ovule and the ability of ovules to act as a surrogate mother. They could derive either from the fusion of two male gametes or from the early natural diplodization of the haploid embryo. This system provides an unparalleled opportunity to shorten the breeding cycle and fix agronomic traits in the homozygous state. Such homozygous true breeding androgenetic seedlings of *Saccharum* X *Erianthus* with hybrid vigour are extremely important for further sugarcane crop improvement.

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