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REVIEW ARTICLE

MESTA-AN ORPHAN CROP: STATUS AND PERSPECTIVES FOR GENETIC ENHANCEMENT FOR MORE PRODUCTIVITY AND VALUE ADDITION THROUGH FRONTIER SCIENCE

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ABSTRACT

Mesta is one of the most important lingo-cellulosic bast fibre crop providing high amount of biomass, however, fibre yield is exceedingly low. It is a multipurpose crop having both domestic and industrial uses and prospects immensely in biofuel manufacturing, carbon trading and subsequently in maintenance of soil fertility. Unfortunately predominant use of synthetic fibres globally reducing its demand drastically. It is a very hardy crop owing to its vigorous growth with considerable drought tolerance. As it is grown as an orphan crop virtually under near zero management no serious efforts has been made to genetically improvise its productivity except some sporadic efforts through exploration to collect diverse germplasm and selection of superior stocks. Meagre efforts have been made to make superior lines even through hybridization. Deployment of modern tools and techniques as embodied in the frontier science like plant biotechnology is virtually nil. The present article narrates the till date achievements made through some systematic scientific interventions and efforts have been made to display diverse avenues through, which this important crop can be improvised endowed with premium quality fibre and other trait enrichment and thus to harness the benefits of this green technology with concurrent attainment of farm prosperity in a remunerative-scale.

INTRODUCTION

Mesta is one of the most popular lingo-cellulosic coarse bast fibre crop having ample economic importance in the public domain, especially in the constrained ecosystems where nothing else is grown. It is mainly grown as an orphan crop on subsistence mode with exceedingly less productivity and production as well. Mesta grows best in well drained sandy loam soil and requires a warm humid climate without excessive rainfall. Among 60 species available only two species are cultivated in India viz. *Hibiscus sabdariffa* and *H. cannabinus,* and belong to the family Malvaceae with chromosome number 2n=36, 2n=72, respectively. It is an annual or biennial herbaceous plant (rarely a short-lived perennial) growing to 1.5-3.5 m tall with a woody base. The stems ranges 1–2 cm diameter, often but not always branched. The leaves are 10–15 cm long, variable in shape. The flowers are 8–15 cm diameter, white, yellow or purple; when white or yellow, the centre remains still dark purple. The fruit is a capsule of about 2 cm diameter, containing several seeds. At present (2012-13) mesta is grown in an area of about around one lakh hectare in country with average productivity of 10-12 q/ha with total production of 106 thousand tones yields (Source: Directorate of Economics, Statistics, DAC, GOI, Agricultural Statistics at a Glance, 2013). Although it is a multipurpose crop, unfortunately it is having very less demand in the domestic and international markets because of variety of demerits, coarse fibre being the prime one. Mesta is cultivated for fibre, paper pulp or as biofuel crop in China, India, Indonesia, Thailand, Russia, Vietnam, Malaysia, Brazil, Argentina, USA and European countries (Webber and Liu 2011).The mesta fibre is invariably mixed with jute to meet the requirements of textile and ancillary industries. The mesta seed oil is used for culinary in various countries and as greasing oil (crude oil for lubrication of machineries) in mesta growing areas of the country. The calyces of mesta have been found as an essential ingredients and base materials in the preparation of jam, jellies, and pickle making. The major constraints of mesta are low seed viability, vulnerability to drought stress, produces coarse fibres, which are not attractive and remunerative in the market. It is a rainfed, marginal crop grown virtually with no care, scientific and technological interventions especially in the form of modern agronomic practices, health care, efficient retting system and use of farm machineries and tools for large scale farming or small tolls for marginal farmers are almost not available. *Hibiscus sabdariffa* L., popularly known as roselle, is a dicotyledonous autogamous, annual or biannual plant having meagre percent of cross pollination. It is a tetraploid species with 2n = 4X = 72 (Akpan, 2000).

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This crop is also grown on diverse soil and agro-climatic conditions world-wide including tropical, subtropical and temperate zones. It is widely distributed in tropical regions of India and parts of Asia, Australia and America (Gomez-Leyva *et al.,* 2008). The *sabdariffa* types are classified into four main groups according to the extent of pigmentation present on the surface of the stem. The colour ranges from full green, green pigmented, green light red and red. The leaves in "roselle" are generally palmate, deeply lobed and alternately borne on the stem. The plants are normally unbranched and attain a height of nearly 3 to 3.5 m with a basal diameter of 1.0 to 2.0 cm. *H. sabdariffa* types are best cultivated in between 10° N and 30° S, where temperature is not less than 10° C and not more than 35° C, together with a rainfall sufficient to give at least 10 inches during each month of the growing period. The *sabdariffa* types in general are not responsive to photoperiodism. In India *sabdariffa* is generally grown in larger parts covering areas from Karnataka to Tripura including Maharashtra, Andhra Pradesh, West Bengal, Bihar, Odisha and Meghalaya.

Kenaf (*Hibiscus canabinus* L.) is an annual plant, native to central Africa, and related to Hibiscus (*Hibiscus hibiscum* L.), okra (*Hibiscus esculentus*), hollyhock (*Althaea rosea*) and cotton (*Gossypium hirsutum* L.) (Scott *et al.,* 1988). Individual plants can grow up to 18ft or more with a few side branches when grown in dense stands. Kenaf is being used as a non-wood fibre crop. The bark, which contains long soft bast fibers, makes up 30 to 40% of the dry weight of the stem. The kenaf plant has an ideal blend of long and short fibres for manufacture of many paper and paperboard products (Grower's, 1989). Most kenaf cultivars are photoperiod sensitive. It is native to Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines especially in home gardens. In India, jute and mesta bear some similarities and are generally grown in larger parts in Karnataka, Tripura, Maharashtra, Andhra Pradesh, West Bengal, Assam, Odisha, and some parts of Bihar, Jharkhand, Uttar Pradesh etc. In Indian languages mesta is calledas *Patwa* (Hindi), *Lal-mista, Chukar* (Bengali), *Lal-ambadi* (Marathi), *Yerra gogu* (Telgu), *Puichchai* (Malayalam) and *Chukiar* (Assam) (Mahadevan *et al.,* 2009).

Apparently, mesta ideotypes should be tall (~2m), less branching or preferably with no branches endowed with premium fibre quality (fine quality long fibres with more tensile strength), should have shiny appearance and compatible for use in the textile industry either singly or as a blending material. Stem diameter starting from bottom to top for should be round/ cylindrical and gradually tapering towards apex. Proportional leaf biomass is must for enhanced photosynthetic) enhanced productivity. It must be tolerant to predominant biotic and abiotic stresses and productivity should be at least double than the present day figure with reduced life span and retting compatible (less time requirement to have clean shiny long fibres). The root system must be more vigorous, since the whole plant weight is dreamt to be more than jute. To fulfill these objectives, a retrospective and perspective is presented below, which is deemed to be possible with existing and emerging conventional and frontier science technologies.

Biotechnological Approaches

Multiple approaches, strategies, tools and techniques are embodied in the biotechnological approaches. Most simply the cell technology approach generally envisages the regeneration of whole plants from single or genetically altered cells through dedifferentiation of organized callus induced from organized explants (plant parts) on artificially synthetic medium fortified generally with plant growth hormones. It is expected to fulfil the demands of market by producing suitable varieties through exploitation of culture induced variation for higher productivity, value addition and in terms of enhanced biotic and abiotic stress tolerances. Development of premium fibre quality, which may open a new vistas in a green fibre crop especially in textile industries, geotextiles, paper pulp and ancillary industries are the major avenues in mesta need to addressed through deployment of biotechnological approaches in a capsule.

Achievements

***In vitro* tissue culture**

In vitro culture is essential to initiate a robust platform for callus formation, shoot regeneration and root induction to produce whole plants in nutshell. Efforts were made *prima-facie* to standardize *in vitro* culture system in mesta involving various hormones, media components, organic adjuvants and various carbon sources for development of successful *in vitro cultured* stocks for whole plant regeneration. There are very limited number of reports on mesta tissue culture as well as transgenic development till date, however, an efficient plant regeneration system is still remaining as the prerequisite to be used for efficient genetic transformation to develop transgenic lines with diverse gene/s. Unfortunately the quantum of biotechnological work especially in cell technology and molecular biology in mesta and allied fibres is meagre and still passing through infancy due to inadequate attention mounted and obviously owing to less *in vitro* culture responsiveness of this crop to produce profuse callus and efficient plantlet regeneration, which is indispensable to develop transgenic plants with added values.

Until now, research has remain focused mainly on the propagation of other ornamental *Hibiscus sp.* such as *H syriacus* (Grange and Loach, 1985; Jenderek and Olney, 1999) *H rosasinensis* (Bertram, 1991) and other Malvaceae family members like *H. acetosella* through culture and subsequent manipulation of shoot apices (Srivantanakul *et al*., 2000), *Gossypium hirsutum* (Ouma *et al*., 2004), *Theobroma cacao* (Minyaka *et al*., 2008). The in *vitro* regeneration systems currently available for roselle is based on meristem culture (Gomez-Leyva *et al*., 2008), by using cuttings (Sie *et al*., 2008) or through use of axillary branching (Sadio, 2000; Govinden-Solulange *et al*., 2009). A direct regeneration protocol targeting seeds was carried out in order to produce some transformed plants (Gassama-Dia *et al*., 2004), but the efficiency was found to be very low. Similarly transgenic

stress tolerant *Hibiscus sabdariffa* plants were produced by a tissue culture independent method using *Agrobacterium tumefaciens* transformation procedure, which resulted in 83.34% GUS positive plants and 60% of transformed plants showed salt tolerance up to the level of 125 mM (Khandker *et al*., 2010).

Somatic embryos were initiated from hypocotyls and cotyledon derived callus of two genotypes of both genus viz. *Hibiscus sabdariffa* var. *sabdariffa* and *Hibiscus sabdariffa* var. *altissima*. Two vegetative propagation methods for roselle softwood and semi-hardwood cuttings from two-month-old plants, which resulted in multiple shoot induction on MS medium supplemented with various levels (0-2.0 mg/1) of BAP/BA (6-Benzylaminopurine) and Kinetin (Govinden-Solulange *et al*., 2009). Good amount of callus induction was reported with cotyledonary explant on MS supplemented with 1.0 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid) and 1.0 mg/l BAP at the subculture 3 by Abeda *et al*. (2014). They also reported an efficient and promising protocol for achievement and enhancement of anthocyanin production from callus culture. *Agrobacterium tumefaciens* based transformation involving a tissue culture independent method was attempted by Gassama-Dia *et al*. (2004) using embryo axes of mature seeds with one cotyledon infected with *Agrobacterium* strain LBA4404 strain containing *gus* and *npt* II gene for plant selection.

Regeneration of shoots from shoot apex as explant on different concentrations of BA (8.87-35.48 µM), TDZ (thidiozuron) (0.91-18.16 µM) and *m*-topolin (8.28-33.12 µM) on MS showed highest number of shoots (33) with the 17.74 µM BA treatment and 16 shoots with 16.56 µM *m*-topolin followed by stem elongation without any growth regulators in dark. Production of roots was observed on 98.6% of regenerated plants with the BA and *m*-topolin treatment (Gomez-Leyva *et al*., 2008). *In vitro* propagation from leaf, node and inter nodal explants through organogenesis was reported by Raaman *et al*. (2013). They showed two morphologically distinct type of calli-creamy yellowish friable calli on MS supplemented with 2,4-D, NAA (Naphthalene acetic acid) and BAP, whereas compact green organogenic calli on MS medium supplemented with various concentrations of NAA and BAP. Shoot formation was obtained directly from nodal explants when MS medium was supplemented with various concentrations of NAA. Calli derived from various explants showed induction of anthocyanin pigments with intense pink colour on MS medium supplemented with 2,4-D and BAP (0.5+2.0 mg/l).

Haploid plants via microspore culture for *H. sabdariffa* var. *sabdariffa* and *H. sabdariffa* var. *altissima*, with three concentrations (1.0, 2.0 and 3.0%) of two sugars (sucrose and glucose) and three explant types (root, hypocotyl and cotyledon) was reported by Rohayu *et al*., (2012). They showed that pretreatment of microspores at 4° C and 35°C for 3 days in the dark and without pretreatment, significantly enhance the percentage of callus induction. Similarly study (Sie *et al*., 2008)was carried out to assess the effect of 14 combinations (NAA/KIN; 2,4-D/KIN; NAA/BA) of plant growth regulators (PGRs) in MS medium and 5 combinations (2,4-D/TDZ) of PGR in Driver and Kuniyuki (DKW) medium involving hypocotyl and cotyledon for callus and somatic embryo formation. The best results for callus induction were achieved with 3% sucrose and the hypocotyl and cotyledon explants, whereas somatic embryos were obtained with DKW medium supplemented with 4 mg/l 2,4-D in combination with 1 mg/l TDZ and 1 mg/l 2,4-D with 0,5 mg/l TDZ. Disadvantages of using TDZ in culture medium showed decrease in shoot elongation, difficulty in rooting of regenerated shoots, vitrification, shoot fascination, etc. These have been reported in many plant species (Huetteman and Preece 1983), similarly Srivantanakul *et al*., (2000)reported that high levels of TDZ exhibited vitrification and fascinated shoots during the process of multiple shoot regeneration, whereas Khatun *et al*. (2003) reported number of shoot production per cotyledon explants could be increased by using surfactant Pluronic F-68, which is comparable to the study of Srivantnakul *et al*. (2000) using TDZ with no harmful effect the regenerated shoots. Thus, the negative effects of TDZ on regenerated shoots could be averted using Pluronic F-68.

Preliminary research on direct shoot regeneration of kenaf using shoot apex as the explant resulted in single or multiple shoot transformation (Zapta *et al*., 1999; Srivantakulu *et al*., 2000). Induction of multiple shoots from young shoots explants of kenaf was achieved by Herath *et al*. (2004) rather than shoot apices. Organogenesis of kenaf via callus culture was found to be not reproducible (McClean *et al*., 1992) and resulted in very low regeneration efficiency. *In vitro* shoot regeneration using leaf explants from varieties like V36 and G4 treated with different combinations of N6, BA and IBA resulted in production of high percentage of healthy callus on MS supplemented with 1.5 $mg.l^{-1}$ BA and 0.5 $mg.l^{-1}$ IBA followed by MS supplemented with 0.3 $mg.l^{-1}$ GA_3 (Giberrlic acid) (68.7 % plant regeneration). All plantlets produced roots in hormone free medium (Samanthi *et al*., 2004). *In vitro* propagation for mass production of kenaf starting from 1 cm micro cuttings of var. Tainung 2 showed most favorable culture medium (Arbaoui *et al*., 2013) for initiation and propagation as MS without hormones for recovery of plantlets from *in vitro* grown explants of kenaf, which could produce 3-8 nodes per explants per month. Rooting was strongly favored by the addition to the medium of MS 0.25$mg.l^{-1}$ IBA and 25 $mg.l^{-1}$ of β- cyclodextrin, which cause the formation of 9-22 roots per *in vitro* grown plants and with average length of 1-9 cm. The rooted shootlets showed 100 % acclimatization under greenhouse condition and there was cent percent survival of the transferred plantlets was observed. Similarly i*n vitro* propagation of kenaf on hormone free MS medium using shoot apex and nodal explants from 15 - days old seedlings without any callus was reported by Abeda *et al*. (2014). However, addition of growth regulators to the MS medium led to decrease in shoot and root induction rates and maximum shoot regeneration frequency (90.5%) were obtained on MS basal medium.

Effect of different combinations and concentrations of growth regulators on callus induction and subsequent morphogenesis from hypocotyls reported to the tune of 100 % callus formation on 2, 4-D alone (0.7, 0.9 and 1.3 mg/l) followed by the media with (0.7 to 1.0 mg/l 2, 4-D, 3 mg/l Kinetin and 0.5

mg/l NAA 1.0 to 2.0 mg/l BA). To induce morphogenesis from calli, both half- and full strength MS with various combinations of growth regulators reported to induce rhizogenesis varied from 10 - 40%, but caulogenesis was only confined to the media containing full strength MS fortified with IBA 0.5 mg/l and GA_3 0.1 and 0.5 mg/l (Karim *et al*., 2011). A rapid and efficient regeneration protocol was established for kenaf using the whole cotyledonary node as explants on MS basal medium by Yang *et al*., (2010), where the effects of the plant growth regulators BA, IAA (Indolacetic acid) and the nonionic surfactant pluronic F-68 on shoot regeneration were evaluated by the orthogonal design L16 (45). They reported plantlet regeneration within two months no morphological difference from those grown *in natura* plants, thus the protocol could be used for kenaf genetic improvement through *Agrobacterium*-mediated transformation in future.

It is observed that in equatorial climates, fibre yield is higher in photo-insensitive kenaf cultivars. In order to develop a rapid screening method, *in vivo* and *in vitro* indices were evaluated by Balogun *et al*. (2013), who screened seven genotypes under natural photoperiod. *In vitro,* stem and leaf explants of genotypes Tainung and V400, which showed contrasting photoperiodic responses *in vivo*, were tested for callus induction at 0 and 12 h photoperiod. Calli were transferred onto differentiation medium at 12 and 9 h photoperiod and numbers of green spots and embryogenic callus clusters were recorded. Both *in vivo* and *in vitro* results showed Tainung as photosensitive and V400 as photoinsenstive. Incubating kenaf callus in differentiation medium in 12 h light and evaluating for greenness was useful in screening for photoperiod sensitivity. Cotyledons with attached petioles in case of kenaf varieties co-cultivated with salt and drought tolerant *Agrobacterium* vector LBA4404 (pBI121CIPKsense) resulted into 80% shoot regeneration with var. HC 2 on MS supplemented with 3.0 mg/l BAP and 0.5 mg/l IAA. About 90 % kanamycin resistant shoots, were found to be GUS positive in var. HC 2 and 80% in var. HC 95. Transformed shootlets were cultured on MS supplemented with different concentrations of NaCl (25, 50, 75, 100 and 125 mM) were able to survive up to 100 mM conc. Non transformed shoots died on salt containing medium. Salt tolerant transgenic shoots were rooted on hormone free MS medium and transferred to soil (Bhajan, 2012). Protoplast from leaf tissue were cultured from six kenaf cultivars on different enzymes and reported the best combination of hydrolytic enzymes as cellulysin (1% w/v; Calbiochem) with macerase (0.5% w/v; Calbiochem), with a 24 hour digestion at 30°C in the dark for protoplast isolation. Electrofusion protocols were developed for kenaf protoplasts testing the range from 1200 to 3000 Vcm^{-3}. A fusion voltage of 2000 V/cm yielded the highest fusion frequency and retained viability above 80 % (Reichert and Liu, 1994). Availability of homozygous parent lines was found to be integral for capitalizing heterosis hybrid breeding in reproducible mode. Production of doubled-haploids is felt essential especially to overcome transsexual barriers to accomplish interspecific and intergeneric hybridization of cultivated crops with wild relatives. The production of haploid plants by anther culture followed by chromosome doubling can be achieved in short period compared with inbred lines by conventional method that requires self-pollination of parent material. Irbrahim *et al*. (2014) used flower buds of appropriate stage of var. KB6 of Kenaf to various pretreatments and different combinations of hormones like NAA, 2,4-D, Kinetin, BAP, and TDZ to induce callus. The best callus induction frequency was found to be 90% in the optimized semi-solid MS medium fortified with 3.0mg/l BAP and 3.0 mg/l NAA.

Different biotechnological tools and techniques like somaclonal variation (SCV), exploitation of which has been reported in many agriculturally important crops (Larkin and Scrowcroft 1981; Mandal *et al*., 1999). Efficient callus induction and prolific plantlet regeneration system is a pre requisite through which plantlets display variations through the process or organogenesis or callus mediated embryogenesis is possible and large number of economically important somaclonal variation are now in vogue. SCV is observed among regenerated plantlets under tissue culture system (Larkin and Scowcroft, 1981). Somaclonal variation is relatively less harsh and without much alternation of parental genetic back up and thus economically important genotypes, varieties can be developed developed with mild variation. Culture under *in vitro* condition enable the genome to accumulate micromutant events, which are generally manifested as somaclonal variation, which sometimes found to enhance productivity and other trait enrichment of course with a risk of appearance of deleterious genetic stocks also. As part of the kenaf improvement program at Mississippi State University, tissue culture is being employed as a means for development of new and improved traits into kenafvia. somaclone development (Reichert and Baldwin, 1996). The regenerates during the process of tissue culture often show altered phenotypes and are coined as somaclonal variation. The exploitation of somaclonal variants, which is a heritable event has been used in various plant improvement programs (Larkin and Scowcroft, 1981; Evans, 1989; Larkin *et al*., 1989; Phillips *et al*., 1994) but there is no hesitation to conclude that success has been sporadic.

Since literature on mesta molecular biology and biotechnology is negligible, referring related works in the same line from the allied fibre crop jute is expected to provide some clues to embark upon genetic improvisation work at least to some extent. Anyhow based on the literature cited above and from the ongoing work on mesta *in vitro* culture the different strategies, tools and techniques, which plausibly be instrumental in mesta genetic modulations are highlighted in a concise way below.

Application of molecular markers in mesta

Application of molecular markers in mesta is at infancy, which warrants more focus in this endeavor. Omals *et al*. (2014) studied genetic relationship among roselle and kenaf accessions using RAPD (randomly amplified polymorphic DNA) markers showed clear separation of both the species except two kenaf varieties, which showed relationship with roselle. Phylogenetic relationship among 73 accessions of kenaf, roselle and their wild relatives from different countries was assessed using 44 ISSR (inter-simple sequence repeat)

and jute (*Corchorus olitorius* L.) specific SSR (simple sequnce repeat) markers. Jute specific SSR markers exhibited high polymorphism and ISSRs displayed high resolving power in kenaf than SSR markers. Number of polymorphic alleles varied from 1 to 5 in case of ISSR and 1 to 6 for SSR markers (Satya *et al*., 2013). The genetic diversity and phylogenetic relationship with 225 SRAP (sequence-related amplified polymorphism) involving 84 varieties of kenaf germplasm from 26 countries and regions around the world has been completed and well documented. High genetic similarity was found among varieties from the same geographic region, resulting from the fact that kenaf is a plant dominated by cross-pollination. Logically SRAP marker could be more reliably used in kenaf varieties identification, studies on genetic diversity and phylogenetic relationship assessment, and germplasm evaluation (Xu *et al*., 2011).

Varietal identification of kenaf had always remain problematic and knowledge on genetic diversity of kenaf varieties is extremely limited, which significantly hindered effective utilization and conservation of the valuable kenaf germplasm. Methods for identifying kenaf varieties followed by their genetic variation with morpho-agronomic characters and RAPD markers among 14 kenaf varieties in Japan showed ample differences (Cheng *et al*., 2001). Morpho-agronomic characters divided the varieties into 3 major groups, but found to be very difficult to identify individual varieties, whereas RAPD clearly separated the varieties. Similar study was carried by Cheng *et al*. (2004) with 23 kenaf and two roselle varieties using morphological character and AFLP (amplified fragment length polymorphism) fingerprinting. The AFLP analysis strongly supports the opinion that kenaf originated in Africa. It also demonstrated that the dissemination of kenaf was from Africa through Asia to Central and North America.

A primary genetic linkage map was constructed using SRAP, ISSR and RAPD with cultivars (Alian kenaf and Fuhong 992) involving F_2 population (180 plants). About 396 loci were used to construct the genetic linkage map with the aid of MAPMAKER/EXP 3.0, and total of 307 loci were grouped into 26 linkage groups that spanned a total map length of about 4924.8 cM with a mean density of 16.04 cM per locus. Those markers were found to be distributed randomly in all linkage groups without any clustering (Chen *et al*., 2011). Similarly Zhang *et al*., (2011) also constructed the high density linkage map with same markers in F_2 population (155 plant) derived through semi-wild var. Ga42 and a cultivar Alain and in respect of 134 loci. The construction of the kenaf genetic linkage map would be one of the interesting the future platform for further genetic studies including mapping of both qualitative and quantitative traits, MAS programme, and in comparative genomics analysis cultivated area of roselle is gradually increasing especially in Egypt for local utilization and exports of calyces, which is having profuse pharmaceutical properties. The calyces extract is a natural colourant an alternative for red synthetic colouring agents in many nutritional, medicinal and industrial purposes due to their high contents of vitamin C, anthocyanins, amino acids and mineral salts. Like cotton seed oil properties roselle seed have a fixed oil, which can participate to cover the need of edible oils in Egypt. However, with the availability of local variety for sepals and seeds they do not spin the expected income, so there is urgent need to introgress novel gene/s from diverse germplasm that would improve the roselle economically. In this endoveour Abou-Ellail *et al*. (2014a) evaluated15 new genotypes for 2 seasons to study their genetic diversity at the levels of yield component characters, genetic parameters etc and RAPD marker was employed to confirm their efficacy as new germplasm, which were reported to be diverse and can be exploited as new germplasm to enhanc the income in Egypt from roselle. Similarly genetic parameters among some selected lines of Sudanese roselle variety in Egypt using morpho-agronomic and ISSR markers were evaluated (Abou – Ellail *et al*., 2014b).

Biochemical and molecular characterization of three roselle types, green (G), light red (LR) and dark red (DR), with RAPD were carried out (Hussein *et al*., 2010) in detail. They studied The total protein electrophoretic profile extracted from their seeds showed inter-individual variation in band density with total protein contents as 46.0, 66.5 and 68.1 mg/g seed, respectively. The total polyphenolic-content and the antioxidant activity of 12 roselle extracts, three coloured types in two solvent systems (aqueous, A and 30% ethanolic, E) and two extraction temperatures (hot, H and cold, C), were also determined by Folin-Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, respectively, which showed discernible differences.

Other biotechnological approaches to be attempted:

Allelmining, Tilling, Transgenic development, Molecular markers, Mapping population development, MAS, Genomics (Structural & functional Genomics) / Transcriptomics / Meatabolomics / Chromosome walking / T5 mutagenesis / Site - directed mutagenesis / (Gene pyramiding / stacking)/ Nanotechnology / mRNAi/ Antisense RNA technology / Methylomes. Owing to paucity of space individual techniques cannot be explained however they have potential in mesta genetic improvement.

A.Development of transgenics for increased productivity and enhanced biotic and abiotic stresses. Through conventional plant breeding, pooling of all desirable genes in a superior stockis practically impossible though spectacular achievements have been made in the past in the endoveour. Since eighties, the era of transgenic research has started very aggressively, which averts the hassels of transsexual boundary and transfer of desirable gene/s from any recipient source, which can be dove-tailed into a large number of a genera to develop a variety of transgenic plants in diverse crops. Even in jute and ramie, successful reports are available. With the help of cell technology, molecular biology tools and techniques, genes can be tailored in desirable ways, for which standard protocols are available and a few protocols are currently available.

Some important tolerant transgenes:

For abiotic stressesgenes like *DREB1, DREB8, CIPK* synthase, *dehydrin, osmotin, glycine-betaine*, *SODs*, bacterial chaperone, Ca- mediated water channel genes, *OSBZ8,*

Rab16A, aquaporin are deemed to be capable to combat drought & *adh, pdc* and *Sub1* can avert submergence stress, *P5CS, SalT* and glyoxylase-1 would be employed for excess salt tolerance.

For imparting resistance to predominant diseases caused by*Macrophomena, Phomasnbderifa, Myrothecium roridium,*– genes like *Xa21, NPR1, chitinase, phloroglucinol, NPRI orthologs, glucanases, plant defensins AFP-Ca* are suitable. To engineer tolerance towards viral diseases sense coat protein, antisense coat protein and antisense Rep genes could be utilized with ease and confidence.

For fibre quality improvement - Silkworm *fibronin, Elastin,* and *PBA* producing genes are suitable.

For insects like Spiral worm (lepidopteran)genes like (*Cry1A(b), Cry1A(c)*), for fleabettle (Coleopteran) gens like *(Cry2, Cry26, Cry37, Cry33, Cry28, Cry1aI)* and for sucking insects like mealybeg lectin genes (snow drop lectin etc) are deem to be useful.

B.Molecular Markers for marker aided selection

Suitable molecular markers should be identified for deployment in MAS to augment biotic and abiotic stress tolerances and to improve fibre quality (major disease like *Macrophomina, Phoma sabdariffae, Myrothecium roridium*, insects like spiral borer, mealy bug and flea beetle etc.). Different kind of marker like RAPD, RFLP, EST-PCR, AFLP, SNP and RAD sequencing – next generation sequencing are potential marker for mesta improvement).Linkage map has been constructed which need to be saturated with more markers for use. Even from a fine map positional cloning of genes would be possible, which can be subsequently introgressed into mesta varieties for trait enrichment. Immense possibility is looming large for adoption of MAS, however no success study is still available in mesta and other fibres. Both foreground and background selection using molecular markers would hasten variety development but since the productivity of transgenics itself is very difficult, the time has not yet come to explore the possibility of using this.

C.Mapping population development:

Mapping population needs to be developed by hybridizing contrasting parents for important characters like disease and insect pest resistance, moisture stress, salinity / alkalinity tolerance and fibre quality traits. QTL mapping for fibre quality genes is essential in diploid and tetraploid mesta. Suitable varieties would be an effective resource for making super fine fibre quality. Association mapping is another lucrative means to tag gene/s governing economically important traits for crop genetic modulation for value addition. Magic population is a new concept to work and tag gene of interest and linkage map, which is having bit similarity with bulk segregation analysis-may be instrumental in unzipping gene location. Furthermore to harness the benefit of genome wide selection (GWS) for exploring most suitable genes in the entire germplasm for genetic enhancement.

D.Omics

Whole Genomics (structural and functional), need to be unzipped to know the genome in details as well as to mimic genes (bio prospecting of novel genes.) Transcriptomics of fibre development, drought tolerance and disease resistance has been initiated and partially done. Microarray may be restored to study expression profiles of diverse genes during fibre development, moisture stress and pathogen challenge. Functional validation of the candidate genes may be done using RNA interference (RNAi) and transgenic approach.

E.Gene pyramiding / stacking

Varieties with pyramided genes need to be developed. It has been observed that in majority of cases, some biotic and abiotic stress are predominant for which candidate genes are already available. By systematic generation wise selection pyramided lines with tolerant genes tolerant to both stresses at HYV background especially in well adaptive popular background in a plausible avenue. Disease and insect resistance genes should be pyramided through conventional breeding and MAS to develop durable resistance. Fibre quality improvement demands extensive research in this endoveour of mesta genetic improvement.

F.RNA interference / Antisense RNA technology / Metabolomics / Nanotechnology:

RNA interference through use of miRNA or siRNA may be used to engineer tolerance in jute against parasitic genes and viruses especially for *Macrophomina* resistance in jute. RNAi – mediated resistance against YMV is possible. Inverted repeat constructs for YMV tolerance and other compatible constructs must be searched out for averting gene silencing. Chromosome walking and T5 mutagenesis, site directed mutagenesis are of immense importance in up and down regulation of many gene/s as and when required. *Agrobacterium*-based gene silencing for trait enrichment demands special attention. Methylome can also be restored for studying gene silencing especially for control of deadly disease *Macrophomina* and Yellow vein mosaic virus, which makes heavy dent in the annual productivity.

G.Nanotechnology

Nanoparticles can provide a new dimension in augmenting productivity through seeds especially by dispening nutrient solution into germinating embryos or by clogging the cell membrane pores which would halt entry and dispersal of disease causing pathogens/insect pests/nematodes etc with special reference to seed borne disease, which causes epidemics very often.

Perspectives

Intensive research work in multidisciplinary mode through deployment of Conventional and Frontier science in synergistic mode would certainly help enable to develop productive varieties with premium quality fibres and product diversification that would help enable to earn huge foreign

exchequer in the days to come if everything is done holistically with highest devotion, dedication and commitment. Suitable package of practices including appropriate health care system and supply of quality seeds at affordable cost in time would help the farmers to augment productivity. Best PHT (Post harvest Technology) and mechanization especially suitable retting techniques are required to get premium quality clean fibres which would be having more selling price both in domestic and international markets to spin more money for achieving robust economy of the country and uplift socio-economics. TOT (Transfer of Technology) through PRA (Preliminary Rural Appraisal) would facilitate skill development to improve productivity. National linkages would strengthen R&D in these natural fibre crops. Private – public and public – public partnership would augment fibre productivity and production as a whole. Development of varietal identity card / barcode would protect Indian mesta genetic wealth from biopiracy. Optimistically we look forward for a revolution in mesta fibre crop husbandry shortly.

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