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Review Article

Propagation Methods of Okra (*Abelmoschus esculentus* L.) and its Application Used in Vitro Plant Regeneration

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Abstract

Okra plant is propagated mainly by using seeds production and also through vegetative part like root, shoot, leaves and apical meristem embryo. its potential self pollinated (autogenous) crop, but considered as cross - pollinated, the extent of cross pollination depend up on cultivar, competitive flora insect population and season. Since okra is an often cross pollinated crop it's difficult for production of pure seed at field. Hybrid seed production of the heterosis is exploited in okra for production of F1 hybrids; generally hand emasculation and pollination are to produce hybrid seed in okra. But hand emasculation and pollination are uneconomical due to less seeds/ fruit. Micro propagation on okra is other option to produce pure seed, the explants like hypocotyls, cotyledonary node and leaf when cultured in MS medium containing NAA or IAA obtained callus formation and root differentiation. Shoot were produced on cotyledon and cotyledonary node explants cultured in a medium supplemented with BA and NAA and roots were also developed from these shoot and plantlets grew normally on transfer to soil. The process no genetic materials exchange the offspring forms single plant, thus result plant identical to the parent plant. Available techniques for the transfer of gen could significantly shorten the time required for conventional breading procedure, to overcome some of the agronomic and environmental problems plant tissue culture has long been recognized an efficient tool for rapid clonal propagation high efficient plant regeneration essential for genetic transformation ex - pant regeneration of okra plant

Keywords: Propagated; MS Medium; NAA; IAA; Okra; Micro Propagation

Introduction

Okra (Albelmosculents) is one of the most widely known and utilized species of the family Maldacena [1], an economically important vegetable crop grown in tropical and sub - tropical parts of the world [2,3]. Okra originated in Ethiopia [4] and was then propagated in North Africa, in Mediterranean, in Arabia and India by the 12th century BC [5]. Okra is known by many local names in different parts of the world it is called lady's finger in England, gumbo in United states of America guano - gumbo in Spanish, GUIBEIRO IN Portuguese bhinidiaa India [6]. In its origin Ethiopia is called Baima. Okra plants are grown commercially in many countries such as India, Japan, Turky, Iran, western Africa, Southern United states [7]. Okra is multipurpose crop due to its various uses of the fresh leaves buds flowers pods stems and seeds [8].

Immature fruits (green seed pods which as consumed as vegetables can be used in salad soups and stews fresh or dried or boiled [9]. Okra seeds have the roasted and ground to form a caffeine free substitute for coffee [10]. To promote the use of indigenous vegetables like okra that have play significant role in mitigate food insecurity alleviate malnutrition in the country. The chemical composition of okra pod per 100 g edible portion 81% 86.1g water energy 144.00 kJ 2.6 g protein carbohydrate 8.2g fat 0.2g fiber1.7g ca 84g Fe 1.2 mg B - carotenoid 185 micro gram. In leaves per 100 g edible portion water 81.5% energy 235 kJ carbohydrate 11.3 protein 4.4g fat 0.6g fiber 2.1g Fe 0.7 g ascorbic acid 59mg B - carotien 385microg riboflavin 2.8mg [11]. The young seed pods are eaten fresh or cooked as a vegetable. The seeds can be used to extract oil. Okra fiber can be used in paper production [12].

Medicinal and nutritional composition of okra

Okra fruits have medicinal values, Alkaline reaction, soothes irritated membrane of the intestinal tract, lowering blood sugar, heal burn and any kind of skin rashes [13], mucilaginous texture soak up unhealthy cholesterol, toxin, mucous waste and clean them from the intestinal tract, acts as laxative that can heal ulcer and may reduce acid reflux, promote good cardiovascular and gastrointestinal health, antioxidant and anticancer [14]. Abelmoschus esculentus is also one the potential natural plant that been used to manage diabetes [15]. There is no much report available on the bioactive properties of A. esculentus despite its wide usage as medicinal plant [16]. Diabetes can be described as increases in glucose in the blood, careful control blood sugar are importance prevents diabetes.

Okra also contains carbohydrates and vitamins [17]. Carbohydrates are mainly present in the form of mucilage [18,19]. Consumption of young immature okra pods is important as fresh fruits and it can be consumed in different forms [20]. Dried okra does not provide any beta carotene (vitamin A) or retinol [21]. However, fresh okra pods are the most important vegetable source of viscous fiber, an important dietary component [22].

Item	Quantity
Water	90.17 g
Energy	31 kcal (129 kJ)
Protein	2.00 g
Total lipid	0.10 g
Ash	0.70 g
Carbohydrate	7.03g
Total dietary fiber	3.2 g
Total sugars	1.2 g
Sucrose	0.40 g
Glucose	0.13 g
Fructose	0.21 g
Starch	0.34 g
Ca	81 mg
Fe	0.8 mg
Mg	57 mg
P	63 mg
K	303 mg
Na	8 m g
Zn	0.60 mg
Cu	0.094 mg
Mn	0.990 mg
Se	0.7 mg

Table 1: Approximate value per 100g edible portion of okra (*Abelmoschus esculentus*).

Source: [23].

It known as powerhouse of valuable nutrients. The fruit is highly protein aces it is good source of vitamin A vitamin C it is low in calories as fat - free and medicinal and industrial value. However, okra has been considered a minor crop and no attention was paid to its improvement in the international research programmed [24].

Ecology and season growth

Okra need temperature above 20°c for normal growth and development [25] germination percentage speed of emergency are optimal at 30 - 35°c. Flower initiation and flowering are delayed with increase temperature (positive correlation between temperature and vegetative nods) [25]. It is short day plant but, it wide geographical distribution (latitude of 35 - 40°c). Flower initiation and flowering are hardly affected by day length subtropical cultivar. The shorter critical day length reported is 12.30 hours.

Biology of okra plant

Somatic chromosome numbers it is Diploid (2n = 130) belong to *Malvaceae* family. Okra is an erect herbaceous annual with green or green with reddish tinge stem. Fruit is a capsule, light green or red in colour with pyramidal oblong broken longitudinally forward 10 - 30cm long and dehiscing longitudinally when ripe.

Floral biology

The okra flowers are 4 - 8 cm in diameter with five white to yellow petals often with a red or purple spot at the base of each petal and the flower within one day the flower structure hermaphroditism and self compatibility. The anthesis takes place at the end of the night the flower is open at down, remained open all morning and close in the middle of the afternoon Flower bud appears in the axial of each leaf above 6^{th} to 8^{th} leaves depending the cultivar. Okra flowers can be very attractive and sometimes used in decorative the rooms [26].

Growth and development

Okra is mainly propagated by seeds and has duration of 90 -100 days. It is generally annual plant its stem is robust, erect variable in branching varying from 0.5mt to 4m in height leaves are alternate and usually palmately five lobed where as the flower is auxiliary and solitary. Okra plants are characterized by flowering continuous but highly dependent up on biotic and a biotic stress. The plants bears first flower one two month after sowing the fruit is capsule and grows quickly after flowering. The greatest increase in fruit length height and diameter occurs during 4^{th} and 6^{th} day after pollination. The okra pod are harvested when immature and high mucilage but before becoming highly fiber production in the fruit starts from 6th days onwards of fruit formation.

Okra breeding methods

Information on the level of diversity for important agronomic traits of okra is limited. Knowledge on genetic diversity and relationships among the okra germplasm may play significant role in breeding programs to improve fruit quality and resistance to biotic and a biotic stresses of okra. Inter - specific hybridization is possible among *Abelmoschus* spp. [27]. This may accelerate diversity and increase gene pool for breeding programs. Diversity within germplasm is critical for okra breeding programs. [28] found differential responses for gas exchange rates, transpiration rate, stomatal conductance, chlorophyll content and leaf water potential in two okra cultivars subjected to two different water regimes. The *A. esculentus* differed from *A. caillei* for 9 out of ten seed characteristics, such as weight, color and oil content. Among 30 African genotypes variation was largely dependent on phenotypic markers [29]. Root knot nematode resistance was also highly variable [30]. Okra flowers are hermaphroditic and heavily self - pollinated. Crosspollination also occurs depending on frequency of pollen transfer by insects [31]. The cross - pollination up to the extent of 4 - 19% [32]. Such out crossing of okra ensures diversity that provides valuable opportunity for further okra improvement and help extended adaptability for local okra genotypes.

Propagation method

Seed propagation (sexual propagation)

Okra plant is potential self pollinated (autogenous) crop, but considered as often cross - pollinated due to its showy corolla and the extent of cross pollination (4 - 19%) in particular place depend up on cultivar, competitive flora insect population and season [33]. Since okra is an often cross pollinated crop an isolation distance of 200 m between cultivar is recommended for production of pure seed. Hybrid seed production of the heterosis is exploited in okra for production of F1 hybrids; generally hand emasculation and pollination are to produce hybrid seed in okra. Emasculation of flowers of female parent is done before anthesis. Emasculated flower are covered with butter paper bags. Pollination is done the next day morning and again covered with the bag. But hand emasculation and pollination are uneconomical due to less seeds/ fruit [34]. Use of mail sterility can be induced by use of chemicals and irradiation.

Advantages of sexual propagation

Simplest and the most economical process among various type of plant propagation. Some plant vegetable species can only through sexual propagation like papaya and tomato. Stronger and disease resistant and long life span method Viral transmission can be prevented this type of propagation and sexual propagation responsible for production of large number of okra crops and too with different varieties and the only propagation process in which result offspring have genetic variation and exhibit diversity of characters from parent crops [3]. This genetic variation have responsible for continuous evolution that keeps production of okra better and offspring. easy storage and transmit ion of seed.

Disadvantages of sexual propagation

Seed take long time to turn plants and time interval between sowing and flowering is longer. Seeding propagated through sexual propagation is unlikely to have same genetic characteristics as that of parent plants. Some plant species do not produce seeds through sexual propagation and hence are unsuitable to propagation for same.

Asexual propagation

Asexual propagation (vegetative propagation)

This process involves production of through vegetative part like root shoot leaves and apical meristem embryo. The process no genetic materials exchange the offspring forms single plant, thus result plant identical to the parent plant.

Advantage of vegetative propagation

Useful trait identically preserved among them. Asexual propagation allows crop that do not seed grow, plant grow through vegetative propagation bear fruit early. The processes are faster than sexual propagation rapid generation crops turn balance the loss. Injured pant can be recovered or repaired through techniques involved in asexual propagation.

Disadvantage of asexual propagation

Diversity is lost in asexual propagation which is the main reason behind occurrence of disease in future plant species, as many crop produce overcrowding and lack of nutrients occurred. It requires special skill and expensive for successful cultivation, shorter life span than those grown through sexual process [3]. And the species involved in this process are less likely to resist pest and diseases.

Micro propagation

Micro propagation studies on okra are very less. Different explants like hypocotyls, cotyledonary node and leaf when cultured in MS medium containing NAA or IAA obtained callus formation and root differentiation. Shoot were produced on cotyledon and cotyledonary node explants cultured in a medium supplemented with BA and NAA and roots were also developed from these shoot and plantlets grew normally on transfer to soil. In hypocotyls explants presence of kinetin or zeatin stimulated callus formation. There was rapid callus induction from explants cultured on MS medium supplemented with (1.0mg/l BA) cotyledonary axial derived callus produce numbers of buds. Micro propagation on okra single node seedling as primary explants was tried to solidified MS medium with 30g sucrose/1 ph 4.7 single node explants were taken from developing shoot and subculture at 4 week interval. Initially plantlets grew and developed well [35].

Regeneration of okra via apical shoot culture system

Studies on micro propagation of Abelmoschus reported previously [36] used a callus initiation medium comprising of MS medium + 40g/L sucrose + 1mg/L2, 4 - D (2,4 - dichlorophenoxyacetic acid 0 + 2g/L phytagel. A simple and reliable protocol for regeneration of okra through somatic embryogenesis from suspension culture. In which embryogenic callus was obtained from hypocotyls explants cultured on media with MS salts Gambro vitamins, 2mg/L, 2,4 - D, 1 mg/L NAA, 25 mg /L Polyvinylpyrrolidone and 30mg/L sucrose [36].

Figure 1: Apical shoot inoculation on Ms Media contain IBA+NAA.

Figure 2: a: Root on MS medium contening IAA+ charcoal 1 g/l. b: Regeneration of apical shoot on Ms Medium containg IBA+NAA.

Concentration of IBA (mg/L)	Number of shoot regeneration (%)	Length of shoot regeneration (cm)
0.25	58.0	1.00
0.50	59.0	1.10
1.00	82.0	2.30
1.50	62.0	1.20
2.00	68.0	1.15
2.50	67.5	1.20

Table 2: Effect of different concentration of IBA on percent shoots regeneration response and shoots length in shoot tip culture of okra when used alone.

Source: [37].

Concentration of NAA (mgIL)	Concentration of 18A (mgIL)	Number of shoot regeneration ('I.)	Length of shoot regeneration (cm)
0.25	1.00	89.0	2.50
0.50	1.00	98.5	3.50
1.00	1.00	92.0	2.90
1.50	1.00	91.0	2.60
2.00	1.00	90.5	2.55
2.50	1.00	93.0	2.40

Table 3: Different concentrations of NAA on percent of shoot regeneration response and shoot length in shoot tip culture of okra.

Source: [37].

Shoot apex culture is an easier method to obtain regeneration plants [38], theoretically the advantage of the shoot apex explants may be obtained from any genotype. Apical shoot regeneration is direct relatively simple need less time to regenerate large number of plants regenerated from shoot apices are true to phenotype with low incidence of semicolon variation and chromosomal abnormalities [39].

In Vitro plant Regeneration and genetic transformation of okra

Available techniques for the transfer of gen could significantly shorten the time required for conventional breading procedure overcome some of the agronomic and environmental problems plant tissue culture has long been recognized an efficient tool for rapid clonal propagation high efficient plant regeneration essential for genetic transformation ex - pant regeneration of okra plant development of new verities. Morphogenic response of hypocotyls cotyledon node and primary leaves were reported [40]. Genetic improvement of crops plant can be achieved by transferring useful alleles at existing loci through conventional breeding or by adding new loci across divers' source through genetic transformation [41].

Okra Ex - plant preparation

Ex - plant material namely hypocotyls cotyledons leaf segments and shoot tips were obtained aseptically grown seedling, seed were washed in running tape water to remove surface particles and then 2%v/v Teepol detergent solution for 4 mint followed by 70% ethanol 1 min and were rinsed in sterilize distil water followed by disinfectant $HgCl_2$ [42]. For 6 - 8 min and finally subjected to rinsed with sterilized distilled water. Surface sterilized seeds were inoculated in test tube containing mist cotton and germinated in the dark. After radical emergence the tube were transfer to growth chamber maintaining at 24^0C with 16/18 h photoperiod (PPFD = 83.µ Em-$^2s^{-1}$ using white fluorescent tube.

Organogenic callus induction

Explants of cotyledons hypocotyls and shoot tip were inoculated MS medium containing 2% w/v sucrose 0.8% ager, and different concentration of IAA, NAA, BAP and 2,4, D 0.5-4 mg/L) [40].

Shoot induction

Ex - plants (hypocotyls cotyledons shoot tip were excised from plant that were two weeks old 6 - 10 cm in height aseptically grown on basal MS medium and subjected to shoot induction by growing them on Ms medium with different concentration of cytokinin's. BAP (0.1-4mg /L, kn (kinetn and zeatin, 1 - 2 mg/L [40]. All tube inoculated at 25°C undere16 h light.

Plant regeneration

Shut bud and shoot derived from well developed calla of cotyledons hypocotyls were transformed to regeneration medium contain MS basal salts B5 vitamins 2% sucrose 0.8 agar were used for shoot bud regeneration. Then since all the cultured plantlets grown in controlled environment need gradual acclimatization for survival of the field. In plantlet at green house control relative humidity and temperature. They had got the result plantlet growth number of shoot per ex-plant 28 - 31 mean of plant growth [42].

Transformation with Agro bacterium Tumefaciens strain EHA105

A tumefaciens strain Eh105 Carr Genetic transformation was carried out with Argo bacterium Tumefaciens carrying the plasmid PBI121 with a selectable marker gene for nptII (neomycin phosphortransferase). Transformed cells were cultured on kanamycin (50 mg/L) and cefotaxime (300mg/L) proliferation of Caius was achieved with complete suppression of Agro bacterium. About 50 - 60% calli showed GUS (b - glucuronidase) expression confirming transformation. thus genetic transformation of okra was successfully achieved by optimizing various parameters for regeneration and agro bacterium infection. the regenerated plant were successfully harden in earthen pots after adequate acclimatization.

Conclusion

Cotyledons were the most effective explants for callus reproduction of roots produced the least. Callus and explants produced somatic embryos up on transferred liquid medium containing 1mg/l 2,4, D. Combination of 1.0 mg/L IBA and 0.5 mg/L NAA were found to be most effective for plant regeneration from apical shoot. Best shoot elongation observed in MS medium supplemented with kinetin 0.5 mg/L. Elongated shoots rooted most effectively in MS medium containing 0.5 mg/L IAA and 1.0 g activated charcoal. The

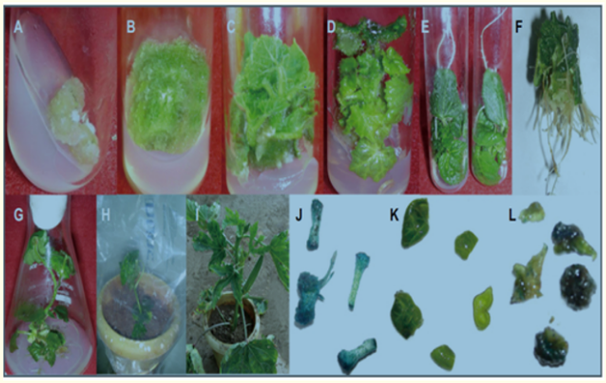

Figure 3: Regeneration of okra var 'parbhani kranthi' whitish compact callus on hypoctyles cultured on MS medium supplemented with TDZ(0.4.mg.l;(B) greenish callus on cotyldanary nodal meristem cultured on MS medium supplemented with BAP(2.0mg/l; © Multiple shoot development from cotyldanary nodal meristem on MS medium with BAP(1.0mg/l);(D) shoot elongation cotyledonary nodal meristem on MS medium with BAP(1.0mg/l); NAA(1.0mg/l)and 0.04mg/l TDZ;(E) Induction of roots from cotyledon nodal meristem on MS medium with BAP(1.0mg/l;(F) profuse in cotyledons cultured on MS medium with NAA91.mg/l ;(G) acclimatization of okra regenerates in culture room (tem 24 ± 20c, 16 hr photoperiod, 60% RH; (H) Acclimatization of okra regenerats okra greenhouse; (I) Transgenic plant; (J) GUS expression [41].

Explant	No of calli incubated	No of gus^{+} calli
hypocotyl	10	6.6
cotyledon	10	5.4
Cotyledonary nodal meristems	10	6.4

Table 4: Transient of gus expression of two explants of okra.

Source: [40]

success of apical shoot culture system of okra was encouraged by acclimatization of the plantlets in the field conditions. Whereas current time modern biotechnology approached use to okra production asexually tumefaciens strain Eh105 Carr. Genetic transformation was carried out with Agro bacterium Tumefaciens carrying the plasmid PBI121 with a selectable marker gene for nptII (neomycin phosphortransferase). About 50 - 60% calli showed GUS (β - glucuronidase) expression confirming transformation. Thus genetic transformation of okra was successfully achieved by optimizing various parameters for regeneration and agro bacterium infection. The regenerated plant were successfully harden in earthen pots after adequate acclimatization.

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