Enhancing Performance of F_1 *Solanum* Interspecifics via Embryo-Culture for Probable Redistribution of their Pharmacological Properties

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Abstract: ***If Solanum species were recommended for treating diabetes and another for cardiac debilities; then there is need to remove them from narrow genetic-base so that working on their further improvement will be enhanced. This trial was carried out at the National Centre for Genetic Resources and Biotechnology, Moor Plantation, Ibadan in tropical rainforest zone, Nigeria. Interspecific crosses were made using five Solanum species; seed were excised from successful crosses and subjected to in-vitro evaluation via embryo-culture of the F_1 generation. Murashige and Skoog (MS) solution used with different treatment combinations indicated significant difference at P=0.05 for all desirable parameters evaluated: especially when low phyto-hormone was added (100 -200nM); hence we so recommend. Callus formation opened insight to fascinating biosynthetic processes and plant evolution, which will remain invaluable for future investigation. In the face of much controversy and suspicion levied on genetic engineering technology, marker assisted selection and mass production of elite plant varieties through tissue culture may remain the preferred choice for years to come.***

Keywords: Solanum, pharmacological-properties, inter-specific, embryo-culture

1. Introduction

The place occupied by members of *Solanacaea* in subsistence farming and for income generation among the peasant farmers of our time is great. This couple with recent finding that eggplants and garden eggs have high pharmacological properties, it also added value to the quality of life for seasoning and preservation of food, it enhances healing process and associated with several anthropological activities. In some localities, eggplants are among those crops set aside for their traditionally value especially during festivals. These herbs and medicinal species has become a cherished diet in ceremonies and traditions older than recorded history (Crake, 1992).

Ayodele, (2002) established medicinal uses of *Solanum aethiopicum* for sedation, vomiting, and against tetanus after abortion. *Solanum macrocarpon* was identified for treatment of boils and throat ailments, while Okot (2004) listed it among useful herbs for treatment of hemolytic anemia. *Solanum indicum* according to Chinese orthodox medicine is used to treat diabetes. It is speculated to be the Biblical Sodom apple. As for *Solanum melongena* it is identified for treatment of several diseases including diabetes, arthritis, asthma and bronchitis, as well as for the treatment of cardiac debilities, neuralgia, ulcer of the nose and cholera. It is also noted as antioxidant, antipyretic and hypolipidermic as reported by Kshayap *et al*., (2003). In spite of these invaluable uses of eggplants, these plants suffer research neglect over the years. Apart from *S.gilo*, among the *Solanum* species and others members of the family as Potato (*Solanum tuberrosum*), have narrow genetic base, which discourages further improvement of these plants (Lu et al. 2004).

2. Literature Survey

A variety of' mechanisms have been described that minimize gene flow between species, which contribute to their reproductive isolation. These may be prezygotic mechanisms, which reduce the frequency at which gametes combine to form a zygote or postzygotic mechanisms, which reduce the viability or reproductive potentials of the hybrid in flowering plants. Earlier studies of *in vitro* regeneration of eggplant were based on culturing cell suspensions (Fassuliotis *et al*. 1981; Gleddie *et al*., 1985), anthers (Dumas de Vaulx and Chambonnet, 1982; Tuberosa et al., 1987) and protoplasts (Jia and Poltrykus, 1981 Saxena *et a*l 1985); Bhatt and Fassuliotis, 1981; Guri and Izhare, 1984; Gleddie *et al*., 1985; Li and Zhang, 1988; Clark *et al.*, 1988). Successful development of morphogenic calli was obtained from isolated microspores, resulting in the production of putative spontaneously doubled haploids (Miyoshi, 1996).

2.1 Factors Influencing In Vitro Regeneration

Several other protocols for plant regeneration via direct and indirect organogenesis have been developed from different eggplant tissues. In those protocols, the regeneration efficiency has been reported to be affected by different factors, such as combination of growth regulators, explant types and genotypes. Most of the organogenic systems reported are based on supplementing culture media with auxins and cytokinins, either singly (Alicchio *et al*., l982; Gleddie *et al*., l985; Sharma and Rajam, 1995) or in combination of the two hormones (Kamat and Rao, 1978; Matsuoka and Hinata,1979; Sharma and Rajam, 1995).

Different sources of explant have been used for the induction of organogenesis in eggplant, including hypocotyls. (Sharma and Rajam, 1995a Magioli *et at*., 1998), leaf (Gleddie *et al*., 1985 Alicchio *et al*., 1982 Mukherjee *et al*., 1991 Sharma and Rajam, l995a Magioli *et al*. 1998), cotyledon stem nodes (Magioli *et al*., 1998) and roots (Franklin and Sita. 2003). The regeneration efficiencies reported in these systems were relatively low (approximately 7 shoots/explant), except in the one described by Sharma and Rajam (1995) who achieved production of 20 shoots/explant in one of the four cultivars

studied. The use of low concentrations (100 - 200nM) of thidiazuron (TDZ) was also reported to induce efficient organogenesis in five cultivars (about 20 shoots/explants) from leaf and cotyledon explants (Magmoli *et al*., 1998; 2000). The effect of different sugars and osmotic conditions has been reported, and the highest regeneration rates were observed in media with low sucrose concentrations (and 22nM) during shoot development. However, the normal concentration of sucrose used Murashige and Skoog (MS) medium (88mM) induced more root development (Mukherjee *et al*., 1991).

Plant *in vitro* morphogenic systems has been used to study the role of polyamines (PA), which were identified as a new class of growth regulators. The effects of polyamines, polyamine precursors and biosynthesis inhibitors during embryogenesis were also reported by Scoccianti *et al*., (2000). A relationship between the spatial distribution of free and conjugated endogeous polyamine and the differential morphogenetic potential within explants have been observed during embryogenesis (Scoccianti *et al*., 2000).

From the review of literature so far carried out no cognizance seemed to have been given as to how the pharmacological properties discovered in different eggplants species could be redistributed among them, considering their different ecological preference/adaptation, in order to make these essential ingredients much more accessible especially to the peasant majority in the sub-Sahara Africa. There is no doubt that problems, prospects and challenges of interspecifics hybridization in *Solanum* species deserves more attention if the evolving genotypes will be of tremendous benefits to agriculture in general and health concern of the society in particular.

3. Materials and Methods

Four of the eggplant species used was obtained from National Institute for Horticulture (NIHORT) Ibadan Nigeria; these include S. *melongena, S. macrocarpon, S. aethiopicum and S gilo;* while *S. indicum* was obtained from a local farmer in Bauchi (where the Hausa people call it-Gauta).

3.1 Treatments

Two hundred (200) seeds of each of the five eggplant species were sown in July 9th, 2005. Six weeks later, 100 seedlings per species were transplanted to the field in the research farm of (NACGRAB). At flowering, the following crosses were made: *S. gilo x S. aethiopicum, S. macrocarpon x S. melongena., S. indicum x S. aethiopicum, S. aethipicum x S. macrocarpon, and S. aethiopicum x S. indicum.*30 crosses in each case.

Twenty-eight (28) days after crossing, two set-fruits from each of the crosses were harvested into five properly labeled beakers, each covered with aluminum foil and taken to the Tissue-culture laboratory. From each of the fruits in the same beaker, seeds were extracted into another smaller beaker. Inside this beaker, 70% ethanol was briskly poured on seeds, shaken and drained off. The seeds were poured into another beaker containing sodium hypochlorite for twenty minutes and rinsed like this twice. This sterilization exercise was repeated for each of the five beakers containing the immature seeds before they were taken to the laminar flow.

Under the laminar flow, second sterilization was carried out. In that the five beakers sealed with aluminum foil, scalpels, medicated cotton-wool and forceps were exposed to the ultraviolet (UV) light for 15 minutes. Then, each F_1 seed was picked with long forceps and embryos were carefully excised using hand-gloves with scalpel. The scalpel was regularly sterilized by flashing it on burning 100% ethanol lamp. The media used was stock solution developed by Murashige and Skoog (MS) medium (1962). Five treatments combinations were: TC_1, TC_2, TC_3, TC_4 and TC_5 containing two groups of growth phyto-hormones as shown in Table 1.

Table 1: Five treatment combinations in mlL^{-1} used F_1 embryos

Media	*Auxin*		*Cytokinin*
TC	NAA	IAA	
TC1	0	0	0
TC2	0.1	0	0.5
TC3	0.1	0	0.4
TC4	0	0	0.05
TC5	0	0.05	0.05

Key: TC= treatment combination; i.e. Mushrage and Skoog (MS) medium with corresponding phyto-hormoes

Two types of growth hormones.(auxin and cytokinin) were used while two types of auxin were used (Naphthalene acetic acid (NAA) and indole-3-acetic acid (1AA) were used, the cytokinin used was Benzlyaminopurine (BAP). The excised embryos were inoculated into 25 x 150mm test tubes containing the media earlier prepared and labeled. Each cross received the five treatments as shown in Table 2, which were replicated four times using Complete Randomized Block Design. The culture was kept in growth chamber at the temperature of 25 $\pm 1^0$C and a constant photoperiod 30. $31Pm^{-1}$ $m^{-2}S^{-1}$ from Sylvania F3bw/ GRD fluorescent lamp.

3.2 Data collection

The following data were recorded 28 days after inoculation: number of calluses formed, green-growth, number of embryo with root. At ten weeks after inoculation, the following data were also collected: number of plantlets with 2-4 leaves (per embryo), number of plantlets with two or more nodes. The above data collected were subjected to analysis of variance (ANOVA) to test for the differences among the effects of levels of growth hormones and for the differences among crosses.

4. Results

4.1 Desirable *in vitro* characters

Table 2a presents means squares for eggplants *in vitro* character under different treatment combinations. Treatment was significant ($P<0.05$) for callus; Table 2a presents means

square for eggplant *in vitro* character under different treatment combinations

sv	df	cal	g.g	rt	lv	n
re	3	4.48	3.0	1.11	3.06	4.05
trt	4	61.80*	93.7**	89.9**	165.42**	155.87**
er	12	0.93	1.50	1.33	1.19	1.51
tt	19					

Key: cal= callus; gg= green-growth; rt= rooted plantlet; lv=plantlet with 2-4 leaves; n= 2 or more nodes.
*, ** significant at p= 0.05 and 0.01 respectively while it was also highly (P<0.01) significant for green – growth, rooting, number of leaves and number of nodes/plant-^{1}. Similarly, Table 2b presents means separation for desirable *in vitro* characters under different growth media. While TC_3 produced significantly more callus, TC_2 produced significantly lower in green-growth. In the same vein, TC_5 produced significantly more in roots, number of leaves and number of nodes formed plant^{-1}

Table 2b: Table of mean separation

TCs	Cal	Gg	Rt	Lv	N
TC1	2.0	4.0	2.7	4.3	4.1
TC2	3.3	3.0	2.7	3.0	3.1
TC3	4.0	4.0	2.6	4.9	4.1
TC4	2.5	4.3	1.8	3.1	2.7
TC5	2.6	4.8	4.3	6.0	5.9
LSD(p=0.05	0.62	0.78	0.72	0.64	0.77
CV(%)	6.64	6.84	8.25	5.15	6.22

Key: abbreviated words as explained under Table 2a. Correlation coefficients of eggplant agronomic character are presented in Table 3.

Table 3: Correlation coefficient

	Cal	G.g	Rt	Lv	N	C
Cal	-	0.12	0.06	0.16	0.03	0.67**
Gg	-	-	0-80**	0.082**	0.83**	-0.58*
Rt	-	-	-	0.79**	0.87**	-.0.43
Lv	-	-	-	-	0.86**	-0.49
N	-	-	-	-	-	-0.44
C	-	-	-	-	-	-

Key: cal= callus; gg= green-growth; rt= rooted plantlet; lv=plantlet with 2-4 leaves; n= 2 or more nodes. *,** significant at p= 0.05 and 0.01 respectively.

Green-growth was positively and highly (P<0.05) significantly correlated with number of roots, leaves and nodes plant-1 with coefficient of correlation 0.80, 0.82 and 0.83, respectively. Rooting was also positively and highly significantly correlated with number of leaves and nodes plant-1. Similarly, number of leaves highly significantly correlated with number of nodes/plant (Table 3).

5. Discussion

Several earlier workers had reported extreme difficulty at obtaining viable seeds from *Solanum* interspecific crosses as characterized by somatoplastic sterility, shrunk seeds and several other evidences of incongruous relationship between the maternal genome and F_1 seeds on it. However, a deviation from these reports was observed in our preliminary investigation in direction that most seeds from eggplant crosses germinated only they did not develop to maturity after germination. Thus, *in vitro* via embryo-culture was consider towards achieving desired goal by enhancing F_1 seeds to develop to full plant using different treatment combinations. The outcome of this indicated significant difference at P = 0.05 for all desirable parameters evaluated. In spite of the fact that treatment combination one (TC_1) the control, did not contain any photyto-hormones added to the stock solution of MS. It was able to generate reasonable amount of desirable characters, whereas TC_2 which had the highest concentration of phyto-hormone failed to match this in most of the desirable parameters evaluated. This therefore supports the findings of Magioli *et al*. (1998; 2000) that the use of low concentrations (100 -200nM) of thidiazuron (TDZ) was reported to induce efficient organogenesis in five cultivars (about 20 shoots) explants from leaf and cotyledon. So also reported by Mukherjee et. al(1991) that the normal concentration of sucrose using Murashige and Skoog (MS) medium (88mM) induced more root development (Mukherjee *et al.,* 1991).

5.1 Calli and green – growth

Callus is the primordial life form with inherent high plasticity for several investigations and subject to manipulations was admitted as one of the parameters of interest in the trial. Although unusual high number of calli as compared with low rate of green – growth differentiation was observed. This appeared to have been influenced by the level of sucrose concentration. Magioli *et al, (*1998, 2000) had earlier reported that the effect of different levels of sugars and osmotic conditions do affect differentiation; in that regeneration rates were observed to be high when low sucrose concentration (11 and 22mM) were used during shoot development. On the other hand, normal concentration of sucrose (88mM) with MS medium induced more efficient root development had been observed. (Mukherjeel *et al,1998)*

5.2 Number of leaves and nodes with 2- 4 nodes per plantlet

The effect of treatment combinations (TC) across blocks were most pronounced with respect to this parameter as elicited by the mean values Table 2b. The performance of TC_5 was outstanding encouraging in sharp contrast with TC_2. It was observed this study that some plots were only able to produce plantlets without leaves, that growth/differentiation did not go beyond the green epicotyl. Interestingly this observation seemed to provide opportunity for better understanding of biosynthesis and fascinating insight to evolution trend in plant kingdom, in that while green – growth is a function of primordial epigenetic differentiation (shoot and root systems); leaves generation is advancement over this leading to specialized morphological differentiation and physiological perfection. It is very important to note that number of nodes per plantlet is fundamental to copping evaluation via subculture, therefore the more the nodes per plantlet the higher the multiplication capacity of the plantlet.

Kamat and Rao (1987) reported that the type and concentration of a given growth-regulator in association to specific genotypes can cause significant differences in the morphogenetic responses of eggplants. For example, using hypocotyls explants induced only the development of ryhzogenic calli in the presence of NAA a-naphthoxyacetic acid (NOA); while regeneration through organogenesis was obtained in the presence of IAA. However no report was made of epicotyls that stopped at green pigmentation stage. Matsuoka and Hinata (1979) also reported that both organogenesis and embryogenesis were observed to respond to different NAA concentrations using the same explants type; but probably ignored this observation too.

Correlation analysis in this study confirms that contamination rate along with any other parameters are significantly negatively correlated, hence no positive relationship. However, callusing maintained near absolute independent relationship with others (Table 3); while parameters such as numbers of plantlets with 2-4 leaves, plantlets with two or more nodes, green-growth and plantlets with varying degree of roots were positively correlated. Essentially it is very interesting that the treatment combinations (Table 1) was able to generate all these desirable characters *in vitro*, they are good enough for multiplication through coppicing for mass production.

6. Conclusion

It is reasonable to conclude that: from this trial it seemed to suggest that those aggressive and hostile genetic interactions earlier reported by several workers as posing interspecific barriers, are now fading away. With the performance of TC_5, it appears that media preparation for embryo-culture or some explant culture of *Solananceae* requires only minute (in micrometer concentration) of phyto-hormone. Hence, the suggestion that for future *in vitro* investigation, the sucrose concentration should be properly moderated for specific differentiation of interest especially for *Solanum* species; whereas if interest is for mass production, it appears that the use of MS stock solution without growth-hormones will be more economical. Strong recommendation is made for investigation at molecular level to unravel the very cause, how far has the interspecific genetic hostility diminished overtime and probable solution/explanation for advancement of biological science. But in as much as mutation and eco-boosted differential genetic expression have contributed to speciation, then the reverse appears to be at play now, as response induced by several years of domestication and co-cultivation of these species. Anticipatorily, result of the present cumbersome interspecific crosses among *Solanum* species will soon be easy as inter-varietal hybridization.

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Benserah making crosses among eggplant (2005)

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