The traditional knowledge systems are perhaps better preserved in the isolation of the Himalayan region or in tribal societies. These knowledge systems need to be studied, documented, preserved – before they are lost under the onslaught of the development projects. As far as I know, except in ethnobotany, medicine and water-harvesting, not much work has been done to study folk knowledge systems in India, or even abroad.

To conclude let me quote Nader[1] again, the belief in the omnicompetence of science has been steadily gaining ground throughout this century in this culture, and operating on a core-periphery model, in the world ... We need not idealize non-western science to make the point that there are different types of knowledge that provide valid truths of use to human kind. If a dominant science silences that knowledge, we all lose. Consider a view that includes the footprints of time: a view of knowledge in which imagination and vision can be openly checked against criticism; the myth of a single science can be seen as myth; the false separation between science and non-science may be considered as a barrier to new thinking .... Ironically, standardization, uniformity, and conformity may not provide the best possibilities for new kinds of science in the long run ....

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SCIENTIFIC CORRESPONDENCE

Multiple shoot formation and *in vitro* fruiting from cotyledonary nodes of *Vigna mungo* (L.) Hepper

Black gram (*Vigna mungo* L. Hepper) is an important grain legume and a good source of dietary protein which is widely cultivated in the tropics, subtropics and temperate zones of the world. The lack of an efficient *in vitro* regeneration system in this legume crop has hindered attempts to tissue culture selection and genetic transformation for crop improvement. The very few available reports on *V. mungo* are hybridization of *V. mungo* and *V. radiata* through embryo culture[1], regeneration from apical meristem[2] and ontogeny of somatic embryos[3]. There is no report about *in vitro* flowering in *V. mungo*. Owing to sex reversal, conventional hybridization is rather difficult in the genus. Further, breeders often face difficulties during hybridization of tropical varieties with the temperate varieties due to the extreme asynchrony of flowering time of temperate varieties. If *in vitro* flower induction of pulses is established, the system would help the breeder for making superior hybrids within a short period. However, in *Arachis hypogaea*[4] and soybean[5] *in vitro* flowering has been achieved, but not mature viable seeds. Hence, in the present report, a highly reproducible and efficient method of nodal regeneration, *in vitro* flowering and pod formation are reported.

For *in vitro* germination of cv. *Vigna mungo* vamban 1, the seeds were surface-sterilized by standard procedures and allowed to imbibe in a rotary shaker overnight. One decoated seed was implanted in each culture tube containing 15 ml Murashige and Skoog[6] (MS) basal medium and allowed to germinate. In all media 3% sucrose was used and gelled with 0.7% bacto agar; the pH was adjusted to 5.8 before autoclaving at 1.4 kg cm^2 for 20 min. Each culture tube was incubated in light–dark (16–8 h) conditions of cool fluorescent light of *ca.* 3000 lux at 25 ± 2°C. For each concentration 20 replicates were kept. The effect of the number of intact cotyledons and the presence of shoot tip on multiple shoot induction was studied by culturing the explants with none, one or both cotyledons intact and with and without the shoot tip. All the different types of explants were cultured on MSB_5 (MS medium + B_5 vitamins[7]) medium with different concentrations of 6-benzylaminopurine (BAP). The most suitable explant and cytokinin concentration were selected. For further production of multiple shoots from the original explant, parent explants were transferred to new culture medium (MSB_5) after 28 days. The percentage of multiple shooting and the number of shoots produced per explant at primary culture and subsequent recultures were calculated. Elongated shoots (3–5 cm) excised from regenerating explants (primary and recultures) were transferred to MSRF (half strength MS inorganic + full strength organic addenda) medium with different concentrations of IBA. The number of roots, flowers, pods and seeds per pod were counted and analysed statistically.

Cotyledonary node explants with or without cotyledons and/or shoot tips elongated after 2 days and gradually induced multiple shoots (Figure 1 *a*) and/or roots according to explant type, hormone and its concentration on MSB_5 medium. The number of attached cotyledons and the presence of shoot tip affected shoot regeneration. The number of shoots produced was greater in the explants with both cotyledons without shoot tip compared to explants with one or no cotyledon (Table 1). Explants with one cotyledon produced multiple shoots from the axis of attached cotyledon, while the other side of the node without cotyledon showed no shoot regeneration but formed callus. This result coincides with an earlier report in *Vigna radiata*[8]. The removal of shoot tip increased the shooting efficiency of the explants. Among the six different types of explants tested, the one with both cotyledons but lacking shoot tip was found to be the most efficient for regeneration.

Figure 1. Nodal regeneration, *in vitro* flowering and pod formation in *Vigna mungo* (L. v. Vamban 1. *a*, Multiple shoots at cotyledonary node; *b*, Reculture of explant after excising pre-formed shoots; *c*, *In vitro* flowering; *d*. *In vitro* fruiting; *e*, Viable seeds from pods produced *in vitro*.

BAP in MSB_5 medium positively influenced multiple shoot formation. MSB_5 medium devoid of cytokinins induced shoot regeneration from the explants with one or both cotyledons in a very low frequency but in the absence of both cotyledons they produced greenish callus. Among the concentrations used, 13.3 µM BAP showed the best response with the maximum number of cultures with multiple shoots (Figure 1 *b*) (Table 1). All the IBA concentrations in MSRF medium resulted in root induction and the efficiency differed with concentrations. Root induction was very quick (7 days) at 2.46 µM and slow (30 days) at 0.049 µM IBA respectively. Callusing was observed before root induction at cut ends of shoots at much higher concentrations (4.9 and 9.8 µM) of IBA.

In the present study different concen-

Table 1. Morphogenetic responses of explants at 13.3 µM BAP

Explant types	Percentage of cultures regenerating multiple shoots[a]	Mean number of shoots/explant	Average length (cm) after 28 days
With shoot tip	4.5 ± 3.5	2.0 ± 0.0	2.5 ± 1.2
With one cotyledon	8.0 ± 2.8	6.5 ± 1.5	3.8 ± 3.1
With both cotyledons	0	0	0
With no cotyledon			
Without shoot tip	6.5 ± 2.6	3.00 ± 0.5	2.7 ± 0.5
With one cotyledon	9.5 ± 1.8	7.63 ± 1.5	4.2 ± 0.5
With both cotyledons	0	0	0
With no cotyledon			

[a]Percentage of 20 replicates.

Table 2. Flowering and pod-forming responses of regenerated shoots at IBA concentrations

IBA (µM)	Percentage of flowering shoots	No. of flowers/plant	No. of pods/plant	No. of seeds/plant	Per cent viability
0.000	0	–	–	–	–
0.049	0	–	–	–	–
0.490	90	7.7 ± 2.2	5.7 ± 1.6	1 ± 0	100
2.460	90	3.6 ± 1.4	3.0 ± 1.1	1 ± 0	100
4.900	0	–	–	–	–
9.800	0	–	–	–	–

trations of MS medium (half strength inorganic + full strength organic, full strength MS medium) were used. Each medium contained 3% sucrose supplemented with different concentrations of IBA. Among the MS media concentrations, flowering response was observed in half strength inorganic + full strength organic MS medium. No flower induction was observed in full strength MS medium. *In vitro* flowering in axillary branches from cotyledonary nodes of Ginseng with half strength nitrogen compounds in MS medium and high sucrose concentration has been reported[9]. Substitution of nitrates in MS medium resulted in flowering from groundnut cotyledon[4]; ammonium was found to promote this flowering. Contrary to the above report, we found that in *V. mungo* half strength ammonium salt induced flowering. In Ginseng, maturation of flower was maximal at 3% sucrose[9]. Our results also suggest that this 3% sucrose concentration is also optimum for flowering and maturation in *V. mungo*. Growth regulators are also key factors in sex expression. Generally auxins induced female flower formation whereas GA_3 enhanced maleness[10]. Even though flower induction was observed when IBA concentration was at 0.49–2.46 µM, we did not come across any unisexual flowers in *V. mungo*. Two to ten flower buds were produced in each cultured plant (Table 2) of which only one developed into a pod while the others abscised (Figure 1 *c*, *d*). Interestingly, irrespective of the IBA concentration, each pod produced only a single viable seed (Figure 1 *e*).

In the present report, establishment of new shoot initials after excising preformed shoots from the existing meristem in subsequent recultures has been investigated which could turn out to be of great use in the transformation of *Vigna mungo*, since *de novo* regeneration systems have been demonstrated to be amenable to *Agrobacterium*-mediated transformation[11]. Nodal regeneration protocols may be further extended to the tissue culture-based selection for stress tolerance. Since shoots are produced from the same explant, nodal culture will more likely provide true-to-type progenies. This protocol can be extended to plant breeding studies and for the purpose of quick flowering and pod formation under *in vitro* conditions.

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