Innovative technologies for quality seed production and vegetative multiplication in forage grasses

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India is facing a shortage of green fodder, dry crop residues and concentrates. The increase in area under fodder crops seems difficult owing to competition from food crops. Hence, utilization of non-cultivable areas for pastures and introducing high biomass producing forages in cultivable lands appear as the viable option. However, the major constraint in revitalizing 85 m ha of denuded grasslands/forest wastelands is the availability of good quality seed and/or planting material. The present article describes the development of technologies for multiplication of seed and planting material in Bajra-Napier hybrid, Deenanath grass and Guinea grass. The *in vitro* rooting of stem cuttings and rapid multiplication of Bajra-Napier hybrid rooted slips using high-density stem cutting nursery were developed to reduce the time and space constraints for multiplication and transport of rooted slips. The innovative defluffing technique helps in extracting the caryopsis from the fluffy seed material of Deenanath grass and significantly reduces the volume. The naked seed so produced is good for pelleting and precise sowing. The *in vitro* maturation of panicles and application of indoleacetic acid during pre-anthesis stage in Guinea grass have resulted in increased seed set and germination respectively. All these innovative technologies are cost-effective, simple and can boost the grass seed and planting material production which otherwise is miniscule compared to the demand. These techniques are in way to solve the myriad problems of grass seed production and multiplication of planting material.

Keywords: BN hybrid, defluffing, Deenanath grass, Guinea grass, high density nursery, *in vitro* maturation.

INDIA with 2.3% share of global geographical area supports nearly 10.7% of the livestock population of the world. Even though it is first in milk production, the productivity of Indian livestock is very low (1538 kg/yr) compared to world average (2238 kg/yr) which can be attributed to malnutrition or under nutrition. To provide sufficient milk to the ever-growing population, there is a need to increase the current milk production of 132 million tonnes (mt) to 160 mt by 2020 and to achieve this, 825 mt of green fodder, 494 mt of dry fodder and 54 mt of concentrates are required[1]. However, at present, India is facing a shortage of 35.6% of green fodder, 10.95% of dry crop residues and 44% of concentrates[2]. Increase in the area under fodder crops seems difficult, because of severe competition from food crops. Apart from vertical expansion from arable lands, utilization of non-arable land area for pastures is a viable option to balance the demand. India possesses nearly 85 m ha of grasslands/rangelands and forest wastelands, which are mostly in degraded state. Revitalizing these denuded grasslands/forest wastelands is the most plausible means to improve the availability of green fodder. Good quality seed and/or planting material is needed to establish the perennial range grasses identified for diverse and harsh climatic situations of arid and semi-arid tropics[3,4]. According to an estimate, only 25–30% of the required quantity of quality seeds is available in India in cultivated fodders and <10% in range grasses and legumes[5]. Production of high volume rooted slips of vegetative propagated grasses and repeated harvesting of grass seeds owing to non-synchronous flowering and maturity makes it highly labour-intensive. The seeds so produced also show poor germination due to non-filling. These factors lead to lesser interest among seed growers in producing grass seeds. Grass seed production is greatly hampered because of indeterminate growth, lack of uniform maturity, seed shattering/shedding, blank seed (fluff with no caryopsis) and long period of seed dormancy[6]. These otherwise required traits in ecological perspective, become major bottlenecks in large-scale seed production.

Among many perennial grasses, Guinea grass (*Panicum maximum*) is well adopted by farmers, because of its multi-cut nature and high green fodder yield (80 to 100 t/ha). The crop is primarily rain-fed; however, under irrigated conditions it gives green fodder throughout the year. It has excellent growing habit with quick regeneration after cutting and good quality herbage with wide adaptability in central India, and in warm humid tracts of eastern and southern coastal India. The seed maturity in Guinea grass varies from plant to plant and from branch to branch within a plant. Even within an inflorescence, different stages starting from anthesis to seed ripening are

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observed. This makes it impossible to realize the full potential of seed production. The manually harvested seeds are characterized by 15% to 30% germination only.

Another important perennial forage for cultivated condition is Bajra-Napier (BN) hybrid (*Pennisetum glaucum* × *Pennisetum purpureum*), which yields up to 250 t/ha/yr green fodder under irrigated condition. There is no seed formation in this interspecific hybrid. Hence, vegetative propagation is the only option for BN hybrid spread. Multiplication and sale of planting material have constraints, such as, high transportation cost due to high volume, damage to the standing crop from where rooted slips are taken out, involvement of high labour cost, etc.

Among annual range grasses, Deenanath (*Pennisetum pedicellatum*) is an important fodder species, because of high early vigour, adaptability to very poor soils and high productivity with minimal input. This grass is also a high seed producer; however, the lightweight small seed enclosed in voluminous fluff leads to difficulties in transport as well as precise sowing in the field. Reducing the volume and extracting true seed for precise sowing is the requirement for large-scale successful usage of this annual forage grass.

Thus, these three important forage grasses have specific problems in seed/planting material production, which need to be resolved to boost their availability for increased production and usage of green fodder in the country. This will also help in rejuvenating the denuded grasslands. In addition to structured formal research, innovations are important and many a time give practical and cost-effective solutions to complicated problems. The present communication in the field of forage seed production presents simple innovations that can solve some practical difficulties, as mentioned above, in forage seed and planting material production and multiplication.

Materials and methods

Five different experiments related to seed and planting material production and multiplication were conducted in three important forage species, viz. BN hybrid grass (*Pennisetum glaucum* × *Pennisetum purpureum*), Deenanath grass (*Pennisetum pedicellatum*) and Guinea grass (*Panicum maximum*) in field and in the laboratory of ICAR-Indian Grassland and Fodder Research Institute (IGFRI), Jhansi.

Stem cuttings with one, two and three nodes were obtained from BN hybrid grass plants for *in vitro* rooting. These cuttings were wrapped in two different media, viz. wet paper towel and newspaper layers with at least one node inside the wrapping. Bundles containing 70 cuttings were prepared using 8–9 layers of paper towel/newspaper along with cuttings. Three replications each were kept at (i) 25°C and 80% RH in a germinator with continuous light; and (ii) at fluctuating temperature (25–30°C) under ambient room condition where the moist state was maintained by regular sprinkling of water on the stem cutting wraps.

The stem/tillers from BN hybrid grass were collected and chopped into pieces of one, two and three nodes called as setts in this communication. A slant basal cut was given to increase the area of contact during planting. The setts were closely planted in upright position with at least one node inside the soil at 5 cm × 5 cm row-to-row and plant-to-plant distance (Figure 1). The stem cuttings were covered with palmyrah leaves or the dry grass or foliage of BN hybrid to protect from heat for one week. Regular water supply was maintained for their establishment and growth.

Deenanath seeds with fluff were passed through common cotton-batting machine used for quilt making after reducing the gap between its rollers. The roller gap was adjusted such that a slight push will allow the seed to pass between the rollers (Figure 2). The naked seed (caryopsis) separated from the fluff was collected just below the roller. The fluff was passed thrice between the rollers to extract maximum available caryopsis. The collected naked seeds were cleaned to remove the inert matter.

Germination of Deenanath seeds (without fluff) was tested in four replicates of 50 seeds each, following a top method according to International Seed Testing Association (ISTA) recommendations[7]. Seeds were kept for germination inside a germinator adjusted at alternating temperature of 20°C for 16 h and 30°C for 8 h with light during high temperature period. The germination percentage was calculated after 10 days, as a number of normal seedlings were produced out of the total seeds kept for germination.

Guinea grass variety, Bundel Guinea 2 (BG 2) was subjected to foliar spray treatment with 25, 50 and 100 ppm indoleacetic acid (IAA). Spraying was taken up on five tillers per plant, in three replications containing five plants each. Simultaneously the same number of tillers were sprayed with distilled water, which were treated as control. Treatments were carried out during booting stage of panicle, twice at 4-day interval. Seeds were collected manually daily after maturity by tapping the panicle on a paper. The harvested seeds were bulked and tested for germination. Seeds were germinated in moist sterilized sand for 8 days, a method standardized for Guinea grass in our laboratory, and the number of germinated seeds was recorded on 4th, 6th and 8th day after sowing. Germination percentage was calculated as total number of seeds germinated out of 100 seeds.

The just emerging panicles of Guinea grass, BG 2, were collected from the field by cutting just above the first node. Five panicles with basal slant cut were dipped up to one cm in 100 ppm IAA and kinetin solutions in 500 ml conical flasks under ambient conditions (Figure 3 *a*). Panicles dipped in water were taken as positive control and without water as negative control. The matured

Figure 1. High density nursery of BN hybrid. ***a***, Transplanting of stem cuttings; ***b***, Stem cuttings with developed leaves and roots, ready for uprooting.

Figure 2. Defluffing of Deenanath grass seed through cotton batting machine.

seeds from the cut panicles were collected manually after 10 days.

The seed filling in treated seed was recorded using non-destructive X-ray radiography method. The seeds were spread in one layer on the plate and exposed to X-rays in MX-20, a self-calibrated radiation unit for 10 sec. The pictures of the seeds were saved for manually counting the filled seeds. In radiography, the bright spots are filled seeds and the remaining are unfilled.

The data was analysed using analysis of variance (ANOVA) with multifactor completely randomized block design according to Snedecor and Cochran[8]. The statistical software SAS 9.2 was used for analysis. Further, the mean values were compared using Duncan's multiple range test (DMRT).

Results and discussion

Planting material multiplication in BN hybrid

In vitro rooting: The propagation of BN hybrid is possible only through rooted slips as the interspecific hybrid is both male and female sterile due to triploid nature[9] resulting in no seed formation for its proliferation. The ability of plant regeneration in BN hybrid was established earlier through several tissue culture protocols[10–12]. By taking cue from those studies, when stem cuttings of BN hybrid containing nodes (with axillary buds) were incubated under high moisture conditions with proper supply of light, they started rooting within a week and were ready for transplanting (Figure 4). Among the stem cuttings (setts), the two and three-node setts showed higher rooting and shooting (91% and 93% respectively) compared to one-node setts (Table 1). Hence, it is better to use two-node setts, as a larger number of setts can be obtained from stem of the same length. Between the two media, i.e. paper towel and newspaper, no significant difference was noticed with respect to rooting and shooting. However, the durability of paper towel is better than newspaper due to its strength and water-holding capacity. After a week of soaking in water, newspaper is easily torn compared to paper towel. Similarly, no significant difference between ambient room temperature and constant temperature in germinator was observed on rooting and shooting of setts (Table 1). This is due to optimum average temperature (26°C) during October, as well as both maximum (31°C) and minimum (21°C) temperatures during that month are similar to alternating congenial temperature range for germination of tropical species, i.e. 20–30°C. Hence, under ideal season with moderate temperature fluctuations, *in vitro* rooting can be attempted even under ambient conditions. Thus, this technique can be used under ambient conditions in suitable seasons without any sophisticated instruments like germinator for providing controlled conditions. The *in vitro* rooting of BN hybrid helps in the production of rooted slips within a short span of one week. As the rooted slips produced in the laboratory are already packed in bundles, it is easy to transport them for long distances without much drying and damage.

High density nursery: The BN hybrid has totipotency in its buds helpful for development of shoot and root. The

Figure 3. ***a***, *In vitro* maturation of Guinea grass using different hormonal solutions; ***b***, Large scale *in vitro* maturation of Guinea grass seed using 100 ppm IAA.

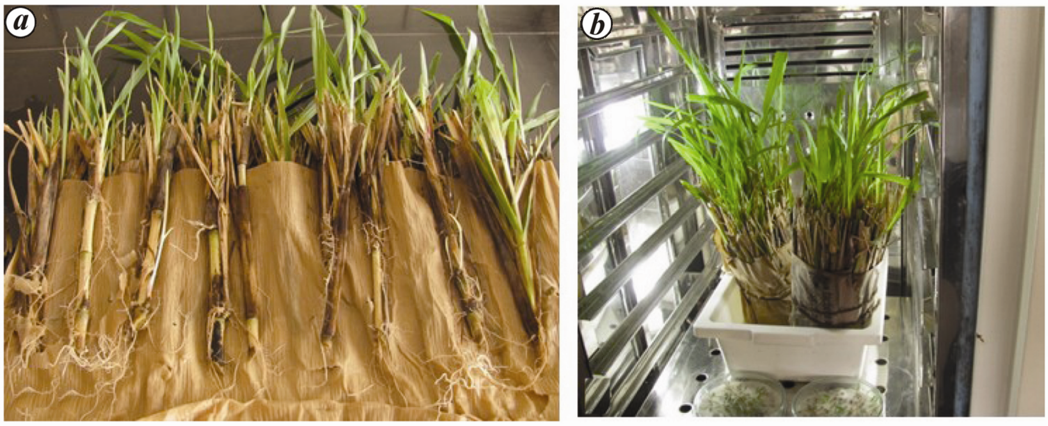

Figure 4. *In vitro* rooting of BN hybrid stem cuttings. ***a***, Stem cuttings with developed roots and leaves after 7 days; ***b***, bundled stem cuttings in germinator.

Table 1. *In vitro* rooting percentage of Bajra-Napier (BN) hybrid stem cuttings with different nodes under different growth conditions

Treatment (no. of nodes)	Rooting percentage of stem cuttings						
	Ambient temperature			Constant temperature (25°C)			
	Paper towel	Newspaper	Mean	Paper towel	Newspaper	Mean	Grand mean
One node	70.37 (57.02)	69.36 (56.39)	69.86 (56.70)	73.06 (58.73)	73.06 (58.73)	73.06 (58.73)	71.46 (57.71)
Two nodes	91.84 (73.40)	89.09 (70.71)	90.51 (72.06)	92.08 (73.65)	91.78 (73.34)	91.93 (73.50)	91.24 (72.78)
Three nodes	93.79 (75.57)	90.37 (71.92)	92.16 (73.74)	94.53 (76.47)	93.67 (75.43)	94.11 (75.95)	93.17 (74.85)
Mean	86.76 (68.66)	83.90 (66.34)	85.36 (67.50)	87.87 (69.62)	87.36 (69.17)	87.56 (69.35)	
	Treatment (Tr)	Medium (M)	Temp. (T)	Tr × M	Tr × T	M × T	Tr × M × T
SEM	0.67	0.55	0.55	0.95	0.95	0.77	1.34
LSD ($P < 0.05$)	1.95	NS	1.59	NS	NS	NS	NS
CV%	3.39						

Figures in parenthesis are arcsine-transformed values.

potentiality of two bud setts of BN hybrid for planting, with one bud outside the soil is reported earlier[13]. Considering this characteristic, a new methodology of high density planting with close spacing of 5 cm using stem cuttings with different nodes was attempted. The shoot buds started transforming into leafy shoots within 10 days and roots emerged after 15 days. The setts were ready for transplanting in 4 to 5 weeks with proper root and shoot. The survival per cent varied among the setts with different nodes. Although the highest survival (83%) was recorded in three-node sett, it is at par with two-node sett (80%) (Figure 5). As stem cuttings were closely planted and young in age, it is easy to maintain them with proper irrigation. In addition, they occupy less space and are easy to uproot and count compared to old tussocks. This high-density nursery can be used for commercial multiplication and sale of BN hybrids in less space and time.

Defluffing of Deenanath seed: The true seeds in the Poaceae family are enclosed in dry indehiscent fruits (caryopsis) which in turn are enclosed in fluff (lemma, pale and glumes) as a natural mechanism for protection and dispersal. However, during seed production in huge quantities, this fluff invariably adds difficulties during handling and transport because of high volume. Deenanath grass (*P. pedicellatum*) consists of involucre with dense bristles apart from lemma and palea resulting in a fluffy covering around caryopsis. This results in reduction of density of seed material and drastic increase in volume. Eight kg of Deenanath grass seed with fluff occupies ~0.1 m^3 volume (equal to an average wheat bag of 50 kg capacity). These seeds with fluff are difficult to sow by broadcasting, as there is high chance of being blown off by wind due to their low density. Each spikelet of Deenanath grass contains 2–3 caryopsis and it is difficult to extract them due to their minute size (2.9 × 0.6 mm). Developing a machine for de-fluffing these seeds has been under discussion since long. In the present experiment, after trying many options for mechanical separation of caryopsis from fluff, we succeeded in devising a more feasible approach to extract large quantities of caryopsis. The commonly used 'cotton batting machine' used for quilt making was used for this purpose after adjusting the roller gap for separation of caryopsis from the fluff. The germination percentage tested in both machine and manually extracted seeds showed no significant difference (Figure 6). Thus, it was inferred that no damage occurred during mechanical seed separation. The quantity of (naked seed) caryopsis separated from one bag of fluff (8 kg weight and 0.1 m^3 volume) was quantified and was found to be 450 g in weight and 0.002 m^3 in volume (Figure 7). This 94% decrease in weight and 98% reduction in volume of seed material is helpful in easy handling during transportation and storage. The naked seed (caryopsis) without any appendages is easy to mix in soil during sowing by broadcasting. In addition, it is easy to prepare seed pellets using naked seed than the fluffed seed with appendages. Incidentally, this newly identified technique is helpful in bulk separation of naked seeds from other similar range grass species, viz. *Cenchrus*, *Chrysopogon*, etc.

Guinea grass production

In the experiment on Guinea grass (*P. maximum*), enhancement of seed germination was targeted. To increase seed germination, exogenous application of several hor

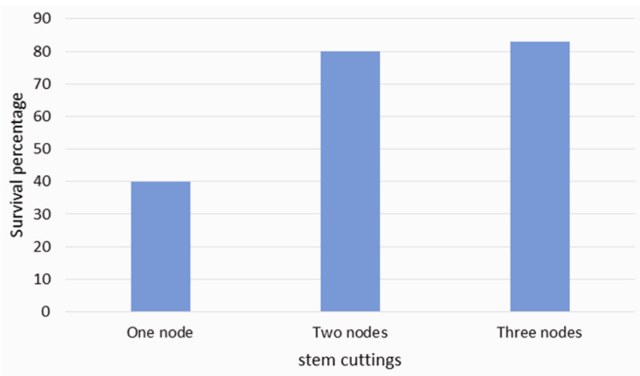

Figure 5. Survival percentage of BN hybrid stem cuttings with different nodes at high density nursery.

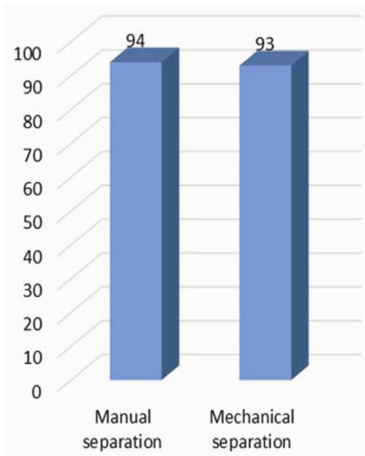

Figure 6. Germination percentage of Deenanath true seed (caryopsis) separated through manual and mechanical means.

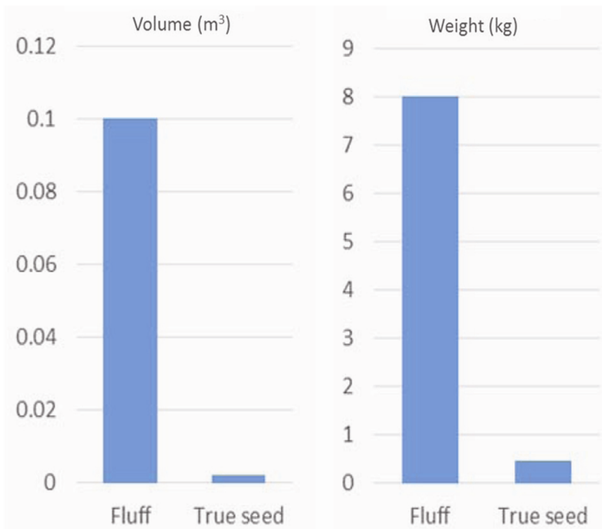

Figure 7. The volume (***a***) and weight (***b***) of Deenanath fluff and true seed (caryoposis).

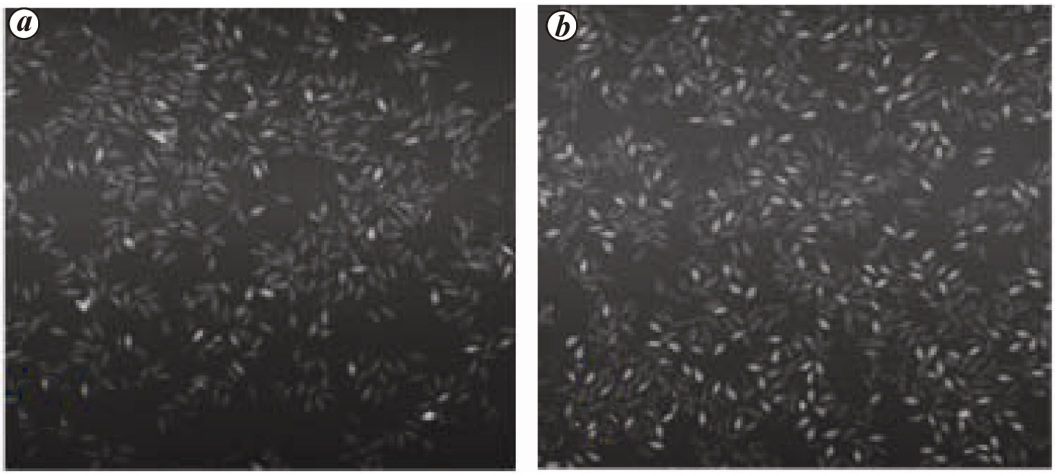

Figure 8. X-ray radiography of Guinea grass seeds (white spots with caryopsis and transparent without caryopsis) collected from cut panicles dipped in solutions (***a***) water (control); (***b***) 100 ppm IAA.

Table 2. Effect of IAA spray on seed germination in *P. maximum*

Treatment	Germination percentage on Day 4	Day 6	Day 8
IAA 25 ppm	18.65 (25.59)	32.98 (35.05)	35.60 (36.63)
IAA 50 ppm	20.32 (26.79)	33.99 (35.66)	38.48 (38.34)
IAA 100 ppm	23.30 (28.86)	40.65 (39.61)	44.98 (42.12)
Control	14.99 (22.78)	22.76 (28.50)	26.83 (31.20)
Mean	19.22 (26.01)	32.42 (34.70)	36.34 (37.07)
	Treatment	Day	Treatment × Day
SEM	0.60	0.52	1.04
LSD ($P < 0.05$)	1.75	1.52	3.04
CV%	5.53		

Figures in parenthesis are arcsine-transformed values.

mones was tested (data not shown). However, IAA application during pre-anthesis stage showed positive results. Further, different doses of IAA (25, 50 and 100 ppm) were applied during pre-anthesis period and germination was confirmed through sand germination test. The germination test of bulked harvested seeds showed significant ($p < 0.05$) increase in germination in all IAA doses compared to control (Table 2). Among the three doses, the highest germination (45%) was observed in 100 ppm IAA on 4th, 6th and 8th day. The germination test revealed that auxin treatment improved the rate of germination as well as per cent germination. This germination enhancement can be attributed to the increased germinable seed, compared to control. The 20% germination standard for Guinea grass according to Indian minimum seed certification standards[14], accords with 27% seed filling as reported by Bahukhandi *et al.*[15]. Thus, application of 100 ppm IAA helped in increasing the per cent germination by enhancing the seed filling in Guinea grass.

Under field conditions, the spikelets of Guinea grass are matured one week after anthesis[16]. In the BG1 and BG2 varieties, the matured seeds shed immediately resulting in loss of valuable seeds. The short span from anthesis to maturity (one week), helped in designing *in vitro* maturation experiment. Varied degree of liveliness was observed in different solutions. In the negative control, i.e. panicles without water, all spikelets dried in a day or two. In the positive control, i.e. panicles dipped in water, the liveliness was observed for 4–5 days. The panicles dipped in 100 ppm kinetin also got dried up even under positive control. However, panicles dipped in 100 ppm IAA solution retained their viability for more than 7 days. It was observed that in cut panicles stigma/anther emergence and withering of stigma, an indication of pollination/fertilization was similar to those events occurring in natural field conditions. After 10 days, the panicles were taken out and the seeds collected by gently tapping, followed by sun drying. The collected seeds were X-ray radiographed to know the filling percentage. IAA (100 ppm) treatment resulted in more than 50% seed setting (Figure 8). These results were analogous to the previous experiment of auxin spraying where germination was 45% in the treated panicles. This further confirms the effect of auxin (IAA @ 100 ppm) on seed filling. Auxin application has been shown to induce fruit-set in many crops[17]. Cell division is a crucial phase in seed formation and auxins play a prominent role in cell division. Starting from ovule development[18] to integument growth[19], auxin signalling is involved in several phases of seed formation. The short period between anthesis to maturity (7 days) coupled with IAA treatment helped in attaining the enhanced seed filling in Guinea grass. The quality seed collection at one place avoiding field loss (shedding), indirectly enhances production and productivity and reduces the overall cost of production. The *in vitro* maturation of cut panicle of Guinea grass for seed production was successfully used for bulk seed production (Figure 3 *b*) and sale at IGFRI.

Conclusions

The new experiments for enhancing seed multiplication/production in three important forage species, viz. BN hybrid, Deenanath grass and Guinea grass, help in reducing the deterrents in large-scale production and increasing the availability of planting material/seed. The high-density nursery and *in vitro* rooting help in faster multiplication of BN rooted slips within a short span of time under field and lab conditions. These technologies lead to ease of transportation and maintenance. The de-fluffed Deenanath seed not only aids in easy storage and handling, but also in sowing either through broadcasting or pelleting. Seed with high germination ability produced by IAA treatment in Guinea grass benefits in the establishment of proper crop stand with less seed rate. The *in vitro* maturation of cut panicles in Guinea promotes production of seed with good filling percentage (50%) and in reduction of field shedding loss. Thus, these simples, but highly useful technologies on large-scale adoption, play a crucial role in enhancing the production and availability of forage seeds.

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