

## Seed Setts, Single Bud Plantlets and Tissue Culture Techniques for Seed Multiplication in Sugarcane and Seed Standard

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### Abstract

Seed multiplication in sugarcane through conventional technique is low (1:10) that's why there is need to increase multiplication ratio through different multiplication technique used single bud, seed setts and top portion i.e. meristmatic region of cane stalk. The notified and released sugarcane varieties those bearing high yield and higher juice quality and also it showed stability and tolerance abilities against biotic and biotic factors under changing climatic scenario. The trueness to variety depends upon its good germination percentage, high genetic & physical purity percentage, vigour and its appearance treated as standard quality seed followed the Seed acted as foundation of Agriculture Technology and modernized much of farming's day-to-day operations also helpful to steady supply of high yielder quality seed, to get high yield. Good quality seed can give up to 15% higher yield in most of the crop. A reviewed upon the advancements of different seed multiplication techniques of sugarcane viz, single bud plantlets (SBP), spaced transplanting (STP), poly bag seedling transplanting, bud chip, sablang or sprouting, rayungan, distance planting, tjoblock, advance vertical seed multiplication, tissue culture and popular conventional methods was done, among them single bud plantlets found the best one. Keeping this above said points in view it is subsidised by the Govt. of Bihar for popularity of SBP among the farmers. Seed standard of sugarcane either in conventional method or tissue culture technique should be maintained so that farmers will get maximum production with quality seed standard.

**Keywords:** Sugarcane; setts; single bud plantlets; tissue culture; seed standard

### Introduction

India is the second most important producer of sugarcane and also consumer of sugar, ranked second in the world while the productivity is low. It is most important industrial crop account for more than 80% of world sugar production while in India more than 90% from it. World's total sugarcane production was estimated at 140,169 lakh tonnes in 2019 and India's share was an average 40,000 lakh tonnes as its contribution 24.47 % of worlds total production (ISMA 2020). In India the productivity of sugarcane is 70 tonnes/ha although there are several notified high yielding and high sugared varieties with productivity more than 80 tonnes/.ha are available which needs to be spread through fast multiplication techniques, it must be maintained seed standard resulted more production. Newly notified sugarcane varieties for each zone or states are already mentioned in the Gazette of India: Extraordinary vide. S.O. 1480(E) dated 1.04.2021, S.O. 500(E) dated 29.01.2021, S.O. 99(E) dated 06.01.2020, S.O. 3220(E) dated 5.09.2019, S.O. 1379(E) dated 27.03.2018 and S.O. 399(E) dated 24.01.2018. The varieties namely CoLk 14204(IKSHU-8), CoPb 98, Co15023,Co 09022 (Karan-12) and CoLk 09204 (IKSHU-7) for Panjab, Hariyana, Uttarakhand, Rajsthan, Central and Western part of Uttar Pradesh(U.P.). CoPb 96, CoPb 91 and, CoPb 95 for Panjab only. The varieties viz, Rajendra ganna-2(CoP 09437), CoLk 12207(IKSHU-6),CoLk 12209(IKSHU-7) and CoP 2061(CoP 06436) for Eastern U.P., Bihar, Jharkhand, West Bengal and Assam while Rajendra ganna-1 for Bihar only similarly CoLk 14201(IKSHU-10) for U.P. only. The varieties namely Co13013, Phule 1001(and Co 09004(Amrittha), CoC 25 for Tamil

Naidu(TN), interior Andhra Pradesh, Telangana, Karnataka, Gujarat and Maharashtra while CoN 13072, CoN 09072, Gujarat Sugarcane 4 (CoN 03131), Gujarat Sugarcane 5(CoN 05071), Gujarat Sugarcane 6(CoN 05072), Gujarat Sugarcane 7(CoN 04131), and Gujarat Sugarcane 8 (CoN 07072), only for the state Gujarat., SNK-632(CoSnK 03632), CoVc 16061, CoVc 16062 and CoVc 99463 only for Karnataka. VSI 12121 and Phule 09057(CoM 12085) for Maharashtra. Kalinga Sugarcane -346 for Odisha, As an exercise of the powers conferred by section 5 of the Seeds Act, 1966 (54 of 1966), the Central Government, after consultation with the Central Seed Committee, is of the opinion that it is necessary and expedient to regulate the quality of the seeds of the varieties. The seeds of above said Sugarcane varieties are notified and must be sold for purpose of agriculture for the States which needs fast seed multiplication to coverage large area. (<https://seednet.gov.in/>).

## Discussions

Seed multiplication in sugarcane takes place through setts (stem bearing three, two and single bud) while for tissue culture purpose upper top portion has been used. Most popular method of seed multiplication is three budded sett while the single bud plantlets raised in plastic tray, a method is now gaining popularity among farmer in sugar mill areas. It is mostly used for gap filling. Single bud plantlets (SBP) raising technology is a viable alternative for quick multiplication of quality seed with replacing the existing deformed seed. During this financial year 2022-2023, Government of Bihar providing subsidy an amount of Rs.30000/- for 10000 SBP to each farmer, which will be allotted to 1100 farmers of Bihar. It will play a vital role for its wide and rapid spread of sugarcane commercial varieties. The details of different seed multiplication techniques of Sugarcane are hereby mentioned below.

### Single Bud Plantlets (SBP)

From cane stalk take out single bud along with upper and lower portion of the nodal region in the ratio of 1:1.5 using sharp cutter device a size of 2 inches. These single bud piece treated with Chloropyriphos @2ml/liter of water + Carbendazim @1g/liter of water for 5 minutes, and incubate in moist gunny bag over night for node priming. Placement of treated seed in tray facing the bud in up and cover it with the prepared soil mixture in the ratio of 2:1:1 i.e. soil, sand and vermicompost, respectively. Provide regular watering in tray and apply the 1% urea solution at 15 day and 25 day after placing the single bud in tray for its proper irrigation and nutrient management.



### ***Benefits of single bud plantlets***

Rapid multiplication of recommend varieties through SBP and spreading of quality seed among farmers as well as factory area due to its high germination percentage. After cutting of all the single buds the remaining part of cane may use for jaggery/vinegar preparation to generate additional income. Low seed rate required seed saving concept, 25000 SBP for one hectare. Transportation facility should be needed, easy to carry which required rack system on trolley (tractor, van) but not more than 4000 while 10000 SBPs carries in truck.

### ***Spaced transplanting (STP) method with single bud***

Either direct sets or seedlings raised in poly-bag nurseries have been transplanted into the field after 50-55 days. STP technique has been developed for synchronisation of tillering and quick seed multiplication ratio from 1:10 to 1:40. It has become popular even in the neighbouring countries. It has contributed in fast spreading of newly evolved varieties at several places. In this method number of 750-1000 plants require for one acre. The require number of settling approx 18000 for this 1.5 to 2 tonnes seed needed either furrow or flat method here rows to row distance maintained 90 cm apart and plant to plant distance of 45 cm apart. Extra stock of settling maintain in the nursery for its further replacement if any plants fails to establish here the space between two settling kept at 30cm apart.

### ***Poly bag plantlets with single bud***

Poly bag plantlets raising technique is similar to STP. Here single bud plantlets are raised in the polythene bags of size 10 cm long and 15 cm width. The soil mixture filled in poly bags with a 1:1:1 proportion i.e. soil, farm yard manure/pressmud and sand, respectively. In one hectare require number of settling approx 25000-30000 for this 1.5 to 2 tonnes seed needed. Here establishment of plantlets in the field is better. By cutting the cane just above node and leaving 8-10 cm of the inter-node below the bud to prepared single bud setts. The plant to plant distance maintained at 45cm apart with small pit dug out and filled with require quantity of phosphatic fertilizer and covered it while planting the plantlets after clipping the green leaves.

### ***'Bud-chip' technique with single bud***

Using a bud chipping machine the nodal region of cane stalk along with healthy buds chipped off from the cane. Further these bud chips are treated properly with fungicide and placed in raised bed nursery or tray/small plastic cup/ small plastic bags and covered with the soil mixture of same proportion as above in case of STP technique. Here the quantity of seed material required is low i.e. only 1 to 1.5 tonnes for one hectare it is the advantage over other techniques because the remaining left over cane after cutting the bud chips can be sent for milling to earn some income. After planting the bud chips either raised bed nursery or plastic tray proper irrigation should be given to maintained moisture and nutrient supply for raising healthy seedlings mainly used in gap filling to maintained the plant population.

### ***Partha Method with setts***

This technique is used to overcome the problems of germination of setts under water logging situation due to rain in july (T.N.) Parthasarthy (1961) developed the method for planting, field divided into ridge and furrow, three bud setts are planted at an angle of 45 degree on ridge leaving at least one bud above the soil. After 5-6 weeks shoot emerge from bud and after another 5-6 weeks when plant attained a height of 20-25 cm the setts are pressed horizontally.

### ***Sprouting or Sablang Method with setts***

In this setts are planted in fertile soil follow shallow or minimum depth of planting, plant to plant wide spacing, adequate dose of fertilization and frequent irrigation. The healthy tillers are separated from the mother plant soon after the developments of its own roots. It can be transplanted separately in the main field. Again same way further more tillers are uprooted and separated from mother plants and planted in the main field. This method is successful used in Java and Cuba.

### *Rayungan Method with single bud*

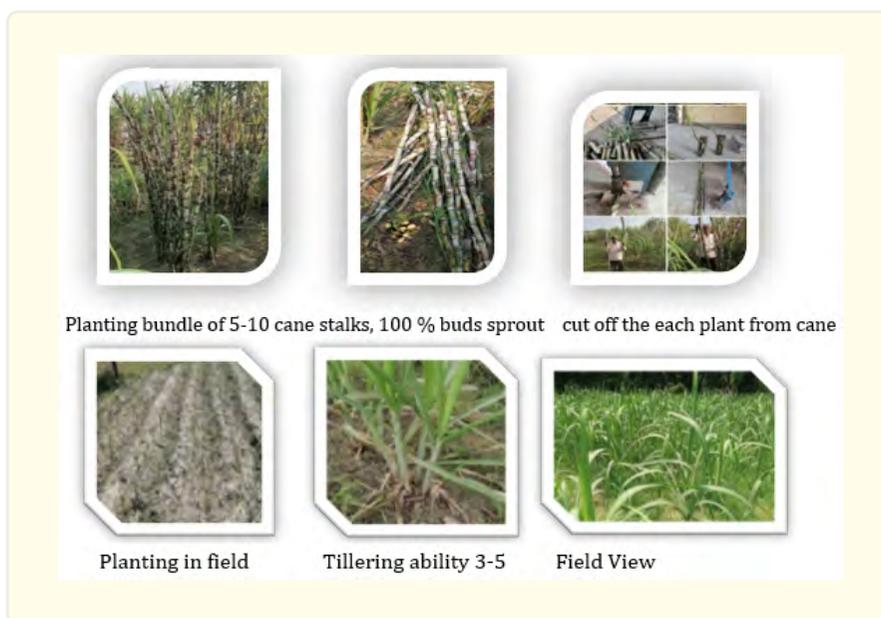
Cane shoot along with single sprouted bud is a term named as Rayungan in Indonesia. In this method for rayungan production a selected portion of field is left during the harvest time. Here cut off the top portion of the standing cane and application of heavy doses of nitrogen and irrigation which result initiation of buds sprout within a week and after 21-27 days all these sprouted buds are separated in single bud setts further transplanted it on ridge. This is not commonly used or adopted by the farmers because of its high raising cost.

### *Tjeblock Method with single bud*

Tjeblock Method is an improve method over the Rayungan due to its proper care for nutrients management and energy to all the buds of the cane stalk while in Rayungan, lower buds have considerable stress to absorbed nutrients. In this method the standing cane stalks are cut off at its half length. Further the half portion of cane planted vertically (Tjeblock) with only one node under the soil for proper rooting. The proper irrigated and adequate fertilizer applied to planted half cane stalk and mother stalks. In due course of time the upper buds of the both (i.e. Tjeblock and mother canes) canes sprouted which are planted by cutting them into setts.

### *Vertical seed multiplication with single bud*

This is an innovative approach, advanced over both viz, Rayungan and Tjeblock method of seed multiplication of sugarcane. Here the standing cane stalk harvested and make a bundle of 5-10 cane stalks further the upper top green leaves cut off from top node. The bundles should be planted vertically so that two nodes of lower portion under the soil for rooting. After planting regular irrigation at least five days interval further limited nutrients should be applied after irrigation. In this vertical seed multiplication technique more than 95 % buds sprout start from upper to lower fashion as per required nutrient supplied to all the buds from the two nodes under the soil. Within 21-25 days almost all the sprouted buds became plantlets with 3-5 leaves on every nodes. This work is doing at SRI, RPCAU, Pusa since last three years, presently the performance of its ratoon is excellent as photograph as planted in circle at SRI, Pusa campus. Progress of single bud plantlets as rayaungan is excellent approx 300 plantlets as tillers are standing while 1400 plants developed from 56 cane stalks of previous year which were developed from 5 cane stalks of next past previous year. The glimpses of this innovative multiplication are depicted in photographic view given below.



In the year 2020 during December 36 sprouted buds (rayaungan) of 5 cane stalks were planted to see its performance. Only 86 plants were developed from 36 in the year 2021 during October out of which 30 cane stalks distributed among the farmers those were participated the training programme as small scale demonstration to assess its multi-location adaptability. Farmers are also planted for its further multiplication and approx 500 plants including tillers. From 56 cane stalks altogether 1500 single buds sprouts were transplanted during winter season and on an average 3-5 tillers are coming from each buds it indicates 4500-7500 plants will be developed. Keeping all the points in view, the approx total number of plants should be 6000-8000 after six month of planting. Presently single bud planning in plastic tray is gaining popularity but cost of production and transportation are the some problems. In this multiplication technique cost of production and transportation will be overcome.

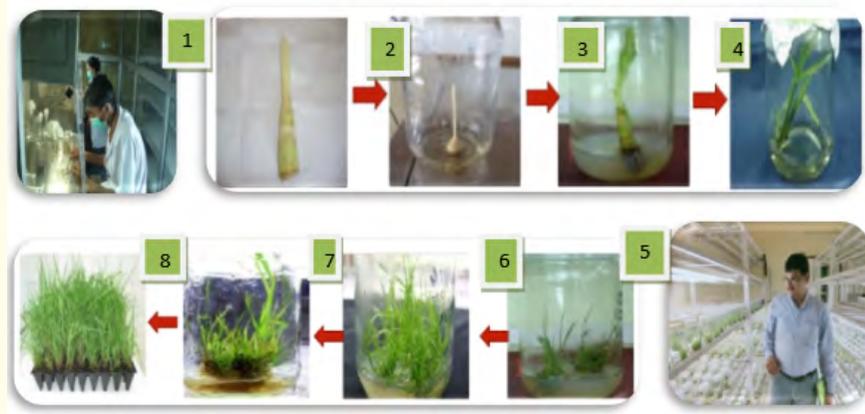
### ***Tissue culture***

It is an useful technique using apical meristem of sugarcane seed cane. Micro propagation of seed cane for rapid multiplication and mass scale of seed production, sugarcane plantlets free from disease. It is useful for faster multiplication of a newly developed high yielder with high sugar bearing sugarcane variety within a short time. Explant used apical meristem, inoculation under aseptic condition inside laminar flow and definite nutrient composition balanced in growth medium. Under controlled temperature and light conditions for 6 weeks incubation tillering production starts from the apical meristem further sub culturing in rooting media. Millions of plantlets cane be developed from one apical meristem within a short period (6 to 8 months) when the plants have the required physiological condition to obtained seed. After the harvest, heat treatment of internodes free from disease through hydrotherapy (Jalaja et al, 2008).

### ***Seed standards for tissue culture plantlets***

The notification of standards for tissue culture plants may take some time as increasing use of tissue culture raised plantlets (TCRP) in recent days has necessitate formulation of standards to maintain its genetic purity as well as quality characters. DBT and VSI, Pune had discussed on this issue and finally a draft was prepared at national level for standards of sugarcane tissue culture (STC). Following are the eligibility requirements/ quality standards to production of STC.

1. Nucleus seed of notified and released variety should be used as explants.
2. Accredited micro-propagation & greenhouse by competent authority.
3. Proper hygienic conditions strictly maintained during micro-propagation, proper potting, planting, irrigation, functional equipments, another laboratory and practices under green house.
4. Hardening of TCRP should be for 6-7 weeks in green house after attain the height of 12-15 cm and bearing 4-5 opened leaves.
5. For proper genetic fidelity molecular test, preferably by Inter Simple Sequence Repeat (ISSR) method.
6. Nodal organization should be the basic material for sub-multiplication as soon as the highest permitted number of passages (as checked by DNA fingerprinting) of shoot multiplication with old cultures has been completed.
7. Developed mother cultures as above is eligible for its further certification while micro propagation facility must be approved.
8. Breeder seed as TCRP tagged with golden yellow label.



**Figure 1:** Sugarcane apical bud or explant (plate 1): Establishment of apical shoot bud (plates 2): Shoot initiation in MS media (plates 3): Shoot elongation (plates 4): Shoot initiation in MS media (plates 3): Shoot Elongation (plates 4): Initiation of multiple shooting (plates 5): Shoot multiplication (plates 6): Rooting (plates 7): Hardening (plates 8).

The following minimum quality standards are essential for greenhouses/poly houses, further hardening of STC as per Indian minimum seed certification standards, The Central Seed Certification Board Department of Agriculture & Co-operation Ministry of Agriculture Government of India New Delhi 2013.

1. Sanitation practices includes proper monitoring of insect and disease and its prevention.
2. Protected greenhouse/poly house and tissue cultured plants.
3. Varieties must be separated by physical barriers and proper tagging.
4. In case of banned viruses, fungus or bacteria identified during testing by an accredited laboratory in such case the tissue-cultured plants must be destroyed and further no any dispatched from the premises of the production lab.
5. STC have been produced as per guidelines by the concerned laboratory/agency which must be certified.
6. The laboratory /agency will follow the labelling procedures of producing STC.



**Green house for hardening**



**Net house for hardening**

### Conventional method

It is most popular method of seed multiplication widely used by farmers. In general farmers follow the planting of sugarcane using three budded setts while two budded setts are popular in limited research area under sugar factories. Here high seed rate required for planting while resulted lower multiplication ratio 1:10. Due to huge seed rate farmers has been facing the problems in transportation. That's why newly developed notified and released variety may takes 5-8 years to its spread in sugarcane cultivated areas. Sugarcane is an annual asexually propagated crop, planted in 5M hectares in India. In India Sugar industry ranked second largest industry next to cotton for generating employment to 50M people directly or indirectly involved for their livelihood that's why it is acted as cash crop. All the steps either different techniques of seed multiplication or general seed production must be followed the classes of seed sanders. There are four classes of seeds starting from nucleus seed further its multiplication in breeder seed under crop breeder/scientist supervision. Another one is foundation seed and again its multiplication in to certified seed; these are adopted for the conventional system of seed production in sugarcane follow the Seed Act.

In India to regulate the quality of seeds for sale, import and export and to facilitate production and supply of quality seeds to farmers the Seed Act was passed during 1966. Further subsequent orders/amendments such as the Seeds (Control) Order, 1983, Seed Bill, 2004 for the minimum seed certification standard, which are achievable by the seed producers and at the same time high enough to meet the needs of the cultivator. There are two parts of minimum seed certification standards first part require the basic and applicable to all the crops under general seed certification standards. The second part requires for specific certification standards which are applicable to only to a crop or group of crops. Under the Seed Act so many numbers of sugarcane varieties cultivated while there is no certification of such varieties by any agency probably due to the bulkiness of seed cane, no any storability of seed cane it makes treble to transport, pack, seal and certify.

To overcome this issue after several round of discussions among the national institution and government the draft was finalized for Specification for Sugarcane Seed Materials and further Standards for Sugarcane Seed Material. Further ICAR in the year 1999 constituted a committee, the committee reviewed the field and seed standards for sugarcane planting material. The Technical Committee of Central Seed Certification Board in October 2001 draft approved the draft after that later notified by the Central Seed Certification Board. The information related to certification of different classes of seeds. Certified seed produced from foundation seed and foundation seed produced from breeder seed while breeder seed produced from either nucleus seed or tissue culture plantlets further the details of responsibility to maintain the purity of seed cane (Karuppaiyan and Ram.,2012). are listed in table.1.

<b>Classes of seed</b>	<b>Seed Source</b>	<b>Place of production</b>	<b>Responsibility for maintaining purity</b>	<b>Certification</b>	<b>Remarks</b>
Nucleus seed	Research station or breeder who developed it. Multiplication by SBP(Single Bud Planting)	Research Centre	Concerned breeder or research Centre	Exempted	The explants for tissue culture derived plantlets are taken from nucleus seed
Breeder seed	Produced from Nucleus seed and tissue culture plantlets.	Research Centre	Concerned Breeder or research Centre	Exempted	Primary nursery is raised from breeder seed
Foundation seed	Produced from breeder seed or tissue culture derived planting material	Govt. Seed Farm, Sugar Factory Farm and progressive farmers' field	Research Centre / State Dept. of Agriculture / State Cane Development Dept. / Sugar factories	Concerned breeder / Expert team / Competent Authority	Supply for production of Certified seed

Certified seed	Produced from foundation seed or tissue culture derived planting material	Govt. Seed Farm, Sugar Factory Farm and progressive farmers' field	State Dept. Of Agriculture / State Cane Development Dept. / Sugar factories	State Seed Certification Agency	Supply for *Commercial seed Production
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\*Commercial seed Production: Produced from certified seed in Farmers' field by Farmers after 3-4 cycles of crop it should be replaced by the fresh lot of certified seeds.

**Table 1:** Seed multiplication of different classes of seed applicable for sugarcane.

### Seed standards followed in Sugarcane

- Age of cane stalk:** At the 6 to 8<sup>th</sup> months age of the crop harvested for seed purpose shall be for planting in tropical states while 8 to 10<sup>th</sup> months should be followed in subtropics. The upper part of cane i.e. floral axis and three lower internodes cut off from seed stalk the rest middle one used for seed purpose.
- Physical purity:** 98% physical purity and maximum permissible limits for lodged canes up to 10% while avoid de-trashing and dry foliage shall be 2.0%. maximum permissible limits. Relaxation may be given such crop in case of water-logged situation up to a maximum of 5% aerial roots / nodal roots. Moisture in seed cane should not be less than 65%.
- Genetic Purity:** A lot of seed material shall be of same variety looking uniform. 85 % Germination ability of buds, no any admixture is permitted and genetic purity should be 100%. Each and every nodes of a seed cane shall bear one viable healthy bud while permissible limits not more than 5%. Swollen buds that projected beyond 1 cm from the rind surface permissible limits 5% of total number of buds.
- Seed Source:** The certified classes will be produced from seed cane whose sources and identity may be assured and approved by the certification agency.
- Land Requirements:** A seed crop of sugarcane shall not be eligible for certification if planted on land on which sugarcane was grown in the previous season. Land of seed crop shall be kept free from sugarcane residues and drainage from other sugarcane fields.
- Heat Treatments:** Foundation stage shall be raised from heat-treated seed cane.

### Field inspection

A minimum of three inspections shall be made as under mentioned in the table as given below.

Particulars	Stage of field inspection	Maximum permissible limit %	
		Foundation seed	Certified seed
1. Off-type	1,2 & 3	None	None
2. Plants affected by designated diseases			
a) Red rot	1,2 & 3	None	None
b) Smut	1	0.02*	0.10*
	2	0.01*	0.10*
	3	None	None
c) Grassy shoot	2	0.05*	0.05*
	3	None	None
d) Leaf scald	3	0.01*	0.01*

3. Plants affected by designated insect pests			
a) Top borer	2 & 3	5.0	5.0
b) Internode borer	3	10.0# None**	20.0 None**
c) Stalk borer	3	20.0+ None**	20.0 None**
d) Plassey borer	3	5.0	5.0
e) Scale insect		None**	None**
f) Mealy bug			
Around 10% affected internodes. * Subject to immediate rouging of the whole clump + Around 0.5% affected internodes. ** In area where the presence of the pest has not been recorded.			

All, the off-types and diseased plants should be rouged out along with roots and destroyed.

Source: Indian\_minimum\_seed\_certification\_standards, 2013.

**Stage-1:** To verify the isolation distance, remove the volunteer plants, designated diseases & pests and other relevant factors for this after 45-60 days of planting first inspection shall be made.

**Stage-2:** To verify off-types plants, designated diseases & pests and other relevant factors the second inspection shall be made at 120-130 days after planting.

**Stage-3:** To verify the age of cane, off-types, designated diseases and pests and other relevant factors. The third inspection shall be made before harvesting i.e. 15 days prior to the harvesting of seed canes.

### Field standards

To avoid mechanical mixture of other varieties, there is need a minimum isolation distance of 5 m for sugarcane seed production fields as recommended for quality seed standard.

Above mentioned during inspection all the off-types and diseased plants shall be uprooted, rouged out and destroyed. Within an area of 5 acres at least 5 counts may be taken while an additional count is taken for each additional 5 acres (Chowdhury, 2007) for sound seed production. Maximum permissible limits for the stripping of dry foliage shall be 2.0%. The seed cane for lodged maximum permissible limits 10%. Seed canes bearing nodal roots under waterlogged areas relaxation maximum permissible limits 5%. Seed moisture should not be less than 65%, germination ability of buds 85%, physical purity of seed cane stalk should not less than 98% and should maintained 100% genetic purity. 5 m isolation distance recommended to separation of fields from other standing cane to avoid mechanical mixture from other varieties of sugarcane.

### Summary and Conclusion

Seed is an essential commodity as per the Essential Commodity Act, 1955 Government of India. For the release of crop varieties the Varietal Release System (VRS) started in India during 1964 with the formation of two committees one at national level i.e. Central Variety Release Committee (CVRC) and another at state level i.e. State Variety Release Committees (SVRCs). The Central Seed Committee (CSC) which was established as per Seeds Act, 1966 under the Ministry of Agriculture, Cooperation and Farmers Welfare in India after that to ensure the quality of seeds on sale and notification of the kinds/varieties are the functions of the CVRC since 1969 which were taken over by the CSC. There are some limitations of sugarcane seed standards due to the bulkiness of seed rate per unit area, problems for transportation as well as its packaging or labelling. Although, the seed cane have short shelf life and its setts should be planted as early as possible after harvest. The Seed cane of 8-10 months age should be prescribed, to follow this seed cane must be planted during

May-July while such planting under North India condition result poor yield. During transport of seed cane quality loss may happen due to this issue the certification agency would be reluctant to certify the crop. Dry foliage of the seed cane and percentage of stripped leaves should not be more than 2% as maximum permissible limit while higher percentage of stripped leaves allowed in case of the some self-detrashing varieties. In sub-tropical India erratic rain and strong winds are frequent during the crop season causes lodging and it may exceed the permissible limit of 10%. In Sugarcane the inspection and monitoring of seed cane for its certification for seed stage is difficult hence in strict sense. The staff of the agriculture department, seed certification agency and sugar mills can be trained for the job to overcome such issues, so that at least the foundation seed and randomly selected certified seed plots are inspected and certified properly. Maintenance of varietal purity and disease management required proper inspection of seed plot. The seed multiplication by single bud plantlet raising in plastic tray require 25000 SBP for transplanting in one hectare for this only 15q of seed cane needed. Seed rate varies variety to variety although low seed rate easy to handle the standard quality and certification. Transportation facility may also easy to carry the SBP with required rack system on trolley (tractor, van) but not more than 4000 while 10000 SBPs carries in truck.

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