

## Successful rescue and field establishment of native banana varieties severely affected by rhizome rot

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### Abstract

Rhizome rot disease caused by *Erwinia* spp. is emerging as a major problem in banana nurseries and young plantations worldwide. Management of the disease is possible only in the initial stages of development. Currently no method is available for rescuing plant material already infected with this pathogen. A total of 95 Nanjanagud Rasabale and 212 Elakki Bale suckers were collected from different growing regions of Karnataka, India. During nursery maintenance of these lines, severe *Erwinia* infection was noticed. We present a method to rescue infected plants and establish them under field conditions. Differences were noticed in infection severity amongst the varieties and their accessions. Field data revealed good establishment and growth of most rescued plants under field conditions. The discussed rescue protocol coupled with good field management practices resulted in 89.19 and 82.59 percent field establishment of previously infected var. Nanjanagud Rasabale and var. Elakki Bale plants, respectively.

**Keywords:** Ney Poovan, rescue technique, Silk banana, soft rot, tip over

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### Abbreviations:

EB: Elakki Bale,  
INIBAP: International Network for the Improvement of Banana and Plantain,  
LYOL: length of youngest open leaf,  
NR: Nanjanagud Rasabale,  
WYOL: width of youngest open leaf

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### 1 Introduction

Bananas and plantains, staple food crops in many tropical countries, are affected by a variety of pests and diseases which can cause catastrophic yield losses (Jones, 2000). Such biotic and abiotic challenges have been the major reasons for initiating crop improvement programmes worldwide (Ploetz, 2004), including the establishment of International Network for the Improvement of Banana and Plantain (INIBAP) in France (Buddenhagen, 1993). Apart from Fusarium wilt, Sigatoka leaf spot, banana bunchy top and other viruses (Sathiamoorthy, 1994), a large number of other diseases have started negatively impacting banana production (Ploetz, 2004). Rot of the rhizome and pseudostem caused by *Erwinia* spp. were considered to be of little significance in the past (Thwaites *et al.*, 2000), but their severity has

increased in recent years in many growing countries including India (Manoranjitham *et al.*, 2010; Singh *et al.*, 2010; Thammaiah *et al.*, 2010; Patel *et al.*, 2011).

Rhizome Rot, also known as Soft Rot or Tip Over Disease is commonly observed during the first 3–4 months after planting under high temperature conditions, especially during late summer and rainy season. It is caused by the bacteria *Erwinia carotovora* (Syn. *Pectobacterium carotovora*) and *E. chrysanthemi*, which are opportunistic residents of banana soils (Thangavelu, 2009). They enter the plant through wounds and spread across fields through water and infected planting material (Ravishankar, 2010). As suggested by the name ‘soft rot’, the plants infected with the pathogen show typical symptoms of rotting in the rhizome, yellowing and subsequent drying of leaves, and breaking of pseudostem at the collar, hence the term ‘tip over disease’.

Symptoms of this disease were first reported from Honduras in 1949, and its widespread distribution is made evident by reports from different regions and countries: Israel (Volcani & Zutra, 1967), Central America (Wardlaw, 1972), Jamaica (Shillingford, 1974), Papua New Guinea (Tomlinson *et al.*, 1987) and India (Singh, 1990). Incidents of Rhizome Rot have become alarming in India in the recent past and for this reason it is the only bacterial disease against which breeding objectives for crop improvement in banana were set (Sathiamoorthy, 1994). The occurrence of Rhizome Rot has been reported from the states of Kerala (Singh, 1990), Gujarat (Singh, 1990; Patel *et al.*, 2011), Tamil Nadu (Manoranjitham *et al.*, 2010), Bihar (Singh *et al.*, 2010) and Karnataka (Thammaiah *et al.*, 2010). Apart from affecting banana fields, soft rot has been reported as a major limiting factor in weaning of micropropagated plantlets (Thangavelu, 2009). The extent of deaths due to soft rot in secondary hardening has been reported to be around 2–5 % (Thomas *et al.*, 2011). Furthermore, *E. carotovora* is known to contaminate *in vitro* cultures (Leifert *et al.*, 1994), causing big losses to commercial tissue culture units.

Nanjanagud Rasabale (*Musa* AAB, Silk banana) is the choicest variety of the state of Karnataka, India. It is an ecotype which has been given the status of Geographical Indication Crop by the Government of India owing to its appealing aroma, attractive bright yellow colour and delicious pulp (<http://ipindia.nic.in/girindia/>). However, this ecotype is highly susceptible to Panama Disease or Fusarium Wilt caused by the fungus *Fusarium oxysporum* f.sp. *cubense* and as a result its production area has been drastically reduced from about 500 ha in 1960 to but a few hectares presently (Venkatachalam *et al.*, 2006). In order to assess the diversity for Panama disease resistance, yield and other agronomic character-

istics surveys were undertaken in growing districts of Karnataka and suckers of putative natural variants were collected.

Elakki Bale syn. Safed Velchi (Ney Poovan group, *Musa* AB), an elite native variety of banana, is popular amongst farmers in the states of Karnataka and Maharashtra, India, as it fetches almost double the price of commercially dominant Cavendish bananas in the domestic market owing to its superior quality fruits with sweet pulp. This otherwise elite variety suffers from the problem of lodging especially in the high wind areas due to taller stature of the plants. To assess the natural diversity for the plant height and other agronomic characteristics, surveys were done in ten districts of Karnataka and desired clones of this variety were collected.

During the period between collection and field planting, the suckers were planted in pots containing sand and were maintained in the polyhouse. As the accessions were from diverse fields (mainly from poorly maintained orchards as main objective was screening for Fusarium resistant lines) even after appropriate maintenance, the plants got severely infected with rhizome rot. Though difficult, the disease is curable in the early stages of infection, if proper control measures are adopted. However, the survival of the plant becomes less likely if the infection is severe and no technique is currently available to rescue the plants which are heavily infected with this pathogen. Efforts were made to save the suckers from the pathogen and this paper presents the method used for rescuing heavily infected banana suckers and establishing them successfully in the field. We hope this report will be of use to researchers dealing with the same pathogen in their germplasm nurseries, so that they may avoid losing their valuable germplasm and to farmers who commonly face this problem during the initial banana field establishment phase. Similarly, it may be a help to nurserymen, who deal with such infections when attempting to multiply banana through macropropagation.

## 2 Materials and methods

The present investigation was carried out in a naturally ventilated polyhouse and the experimental fields of the Department of Horticulture, University of Agricultural Sciences, Bengaluru, India. The experimental field is located in the eastern dry zone of Karnataka (12°58' N; 77°35' E) at an altitude of 930 meters above mean sea level.

### 2.1 Germplasm collection and nursery management

Exploration was carried out from January 25<sup>th</sup>, 2011 to March 25<sup>th</sup>, 2011 in eleven districts of the state of

Karnataka. In case of Nanjanagud Rasabale (NR), extensive field surveys were carried out in the Mysore and Chamarajanagar districts of the state. Clones showing various degree of resistance to Panama Disease were selected and the maximum possible numbers of suckers were procured from the selected plants. For collection of Elakki Bale (EB), dwarf stature, resistance to Panama Disease and good agronomic parameters were the selection criteria and collection was done in ten districts: Bengaluru (Urban), Bengaluru (Rural), Chitradurga, Davanagere, Hassan, Kolar, Mandya, Mysore, Ramanagara and Tumkur.

Collected suckers were brought to the polyhouse and washed with water to remove the adhering soil particles. Dried leaf sheaths and roots of the suckers were removed using a sharp knife prior to soaking in 0.2 % Bavistin solution for 20 minutes. Treated suckers were then planted in polypropylene pots containing sterilized sand. Pots were maintained in polyhouse before planting in the field. After one month, a plant was observed to have bacterial rot symptoms which were confirmed after its examination and within the period of one week, the number of infected plants increased. Soon after the incidence was noticed, the plants were drenched with a solution containing copper oxychloride (0.3 %, Blitox, Tata Rallis, Mumbai, India) plus 600 mg L<sup>-1</sup> Krocin AG (streptomycin sulfate 9 % plus tetracycline hydrochloride 1 %, Krishi Rasayan, India) and the treatment was repeated at weekly intervals for three weeks, as the spread increased in intensity resulting in the loss of many accessions.

## 2.2 Rescue method

As the severity of the disease was high and initial control measures were not effective, plants were given treatments of methoxy ethyl mercuric chloride (MEMC, Bagalol, United Phosphorus Limited, India). For treatment, the infected tissue was scooped out until healthy white rhizome tissue was revealed. Care was taken that no rotten portion remained on the rhizome surface. Such shaved rhizomes were treated with 0.2 % MEMC solution for 5 minutes and were planted in new pots containing a sterilized mixture of red soil and coir pith in equal proportions. The suckers had little or no roots and the size was reduced to a piece of about 2.5 cm thickness of healthy tissue. Plants were watered judiciously as and when required. To promote root formation and plant growth, plants were sprayed twice with mono ammonium phosphate (0.2 %) at weekly interval.

## 2.3 Field planting and aftercare

After 45 days of treatment, plants were transplanted to the field. Field was prepared by ploughing twice and levelling. Pits of 60×60×60 cm were dug at the spacing

of 2 × 2 m and filled with one kg vermicompost, 15 kg farm yard manure and top soil. As a precautionary measure, holes were sterilized with one litre 0.2 % MEMC solution, one day prior to transplanting. To provide protection against nematodes, 50 g of Tataburan (carbofuran 3G) was added to and mixed into the soil in the planting holes before planting. Plants were irrigated just after planting and subsequent operations were performed following the package of practices developed by the National Research Centre for Banana, Trichy, India (<http://www.nrcb.res.in>).

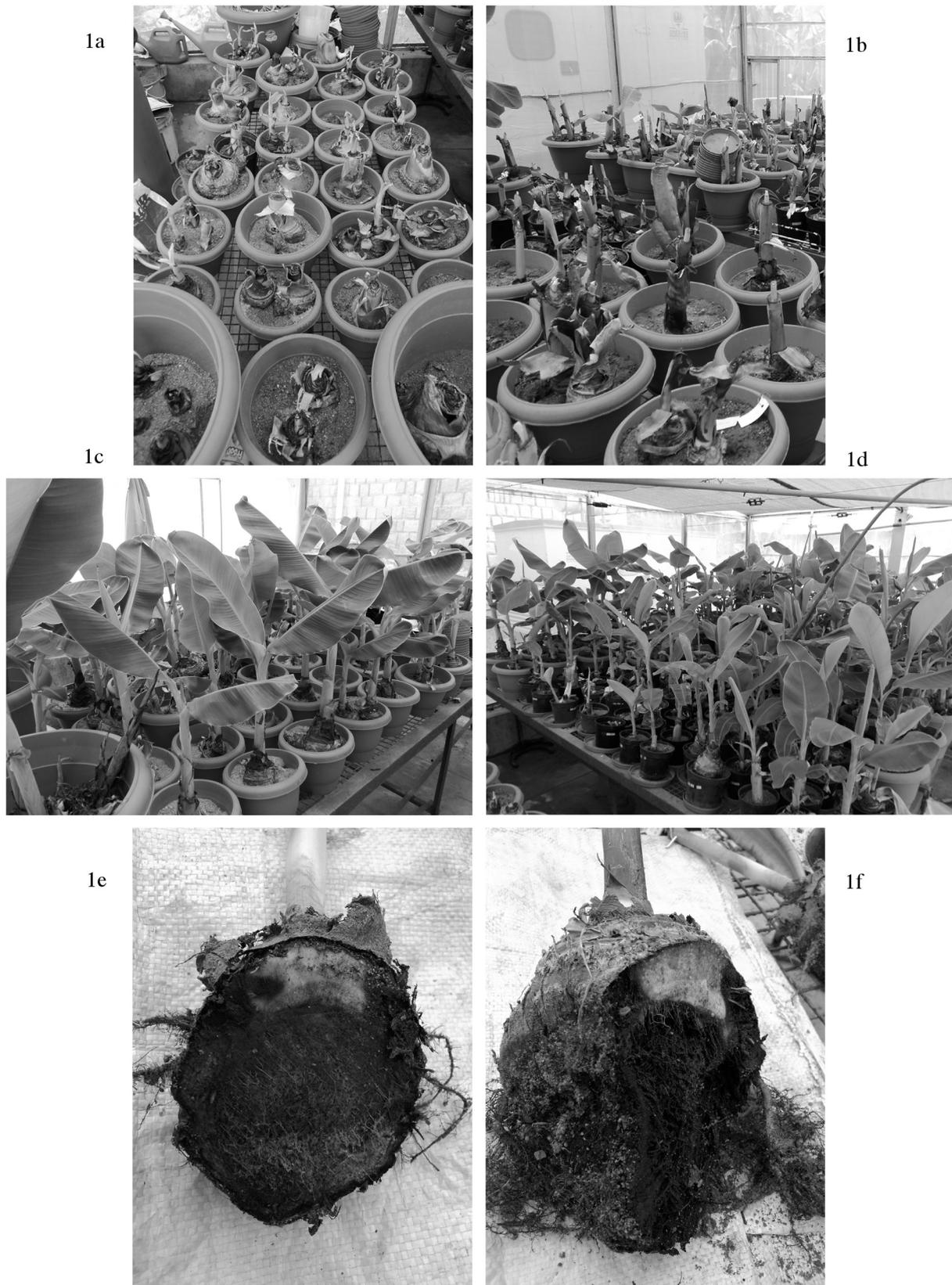
## 2.4 Recording observations and statistical analyses

Survival and field establishment of rescued plants was calculated based on the number of plantlets that survived the first four months after transplantation. No *Erwinia* disease was observed in the field thereafter. Parameters such as plant height (cm), collar diameter (mm), number of suckers, number of leaves, length (cm) (LYOL) and width (cm) (WYOL) of youngest open leaf were recorded at two months intervals: two and four months of planting, as this is the most crucial period in plantlet establishment. Infection resulted in unequal numbers of replications in most of the accessions; hence the mean of the available plants was taken into consideration for comparing the growth performances. Paired t-test was done using the Web Agri Stat Package (WASP, v. 2.0, ICAR Research Complex for Goa) to compare the growth parameters between establishment (two months after planting) and post establishment phase (four months after planting).

## 3 Results

### 3.1 Development of disease in nursery

Suckers of all accessions in both varieties started sprouting 10–15 days after planting in pots (Fig. 1a and 1b) and good growth was noted within one month (Fig. 1c and 1d). The symptoms of rhizome rot started about four weeks after planting in pots with yellowish brown blighting from the leaf margins, progressing towards the midrib. The affected portion started drying up and almost all mature leaves showed similar symptoms. During this initial phase, the rhizome appeared to have a hard surface but showed typical dark brown rotting when excavated from sand and cut crosswise (Fig. 1e). As the disease progressed, the rotting extended to the whole rhizome except for a bit of healthy tissue near base of the plant. The rotten portion looked like delicate black threads and was moist (Fig. 1f). During this phase, the pseudostems broke at the collar region and were unrecoverable.



**Fig. 1:** Development of disease in nursery: (a & b) sprouting of NR and EB suckers, respectively; (c & d) growth of NR and EB suckers; (e) rotting of the rhizome due to *Erwinia* sp.; (f) advance stage of disease development.

### 3.2 Efficiency of rescue methodology

Data on the number of plants that died due to Rhizome Rot at each stage is presented in Table 1. Mortality was divided into post infection loss due to *Erwinia* in polyhouse and post treatment (rescue) loss under field conditions. Different accessions of NR showed various degrees of susceptibility to rhizome rot. Out of a total of 95 suckers from 27 different accessions, 22.11 % (21 suckers) died in the nursery, wiping out all the replications of accession NR 1. About 14 accessions showed tolerance to rhizome rot: no deaths were reported from these lines, even after infection. Thirteen of these lines maintained tolerance and recovered on transplanting in the field with 100 % establishment. The overall post treatment survival rate under field conditions was 89.11 %.

**Table 1:** Sucker losses due to rhizome rot under different conditions and field establishment of the rescued plants.

Parameters	Clone	
	NR	EB
No. of suckers at collection	95	212
Suckers lost in nursery due to <i>Erwinia</i>	21	126
Suckers lost (%)	22.11	59.43
No. of suckers planted in the field	74	86
No. of plants in field 120 DAP	66	71
Field establishment (%)	89.19	82.59

The losses of EB in nursery stage was 59.43 %, resulting in the complete loss of thirty one accessions. Suckers of eleven accessions, though infected, survived the nursery stage. However, one of these lines was killed off by the pathogen under field conditions, probably due to the systemic presence of the pathogen and the shock of transplantation. The rhizome rot survival rate of EB was 82.59 %.

### 3.3 Post-rescue field establishment

In order to study the effect of the rescue protocol on subsequent establishment of plants in the field, observations were recorded on different growth parameters two and four months after planting. Data is presented in Tables 2 and 3. Main objective was to find out the growth differences between establishment (Fig. 2) and post establishment phases (Fig. 3). The comparison of these phases using paired t-test in NR revealed highly significant differences for parameters such as plant height and collar diameter. The number of suckers were also found to be significantly different, but were observed to be less in post establishment phase than in the establishment phase. Length of the youngest opened leaf

increased significantly, whereas number of functional leaves on the plant and the width of youngest opened leaf remained non-significant (Table 2). Seven accessions (NR 8, NR 12, NR 14, NR 18, NR 19, NR 21 and NR 24) showed excellent establishment and growth during post establishment phase.

In case of EB, highly significant differences were recorded for parameters such as plant height and collar diameter, number of leaves, and length and width of youngest opened leaf. Two to four fold increases in plant height was observed amongst the accessions between establishment and post-establishment phases (Table 3). However, apparent variability was observed among the accessions. Collar diameter also followed the same trend and significantly improved in two months. There were no significant differences in number of suckers, though. The reason might be the smaller size of the chopped rhizome in which only a few lateral buds remained. Many accessions did not produce any suckers within the first four months after planting. This might be due to genetic differences between them. Cumulative count of leaves improved in a highly significant manner where the range of functional leaves increased from 6–10.7 to 8–14 leaves/plant. Length of youngest opened leaf also increased over time, which might be due to increasing petiole length of the succeeding leaves. Due to normal root growth, plant metabolism also improved and hence, leaf length as well as width increased in this formative phase of plant growth.

## 4 Discussion

The name soft rot derived from the characteristic soft decay of the fleshy tissues of the rhizome of infected bananas. Banana, like most tuberous and bulbous plants, has higher carbohydrates in the rhizome tissues and these storage organ tissues have cells in semi dormant condition. When such tissues get infected with *Erwinia*, they become soft, watery or slimy in consistency and with the progress of the rot, water exudes from the affected region (Walker, 2004). These symptoms were also noticed during the course of present investigation. The softening is mainly caused by the action of pectic enzymes on the storage tissues (Mehrotra & Aggarwal, 2003).

Literature review suggests that this disease has not been recorded in Ney Poovan nor in Nanjanagud Rasabale, though rhizome rot has previously been reported in different banana cultivars: Gros Michel (Stover, 1959), Nendran (Singh, 1990), Dwarf Cavendish (Singh, 1990; Patel & Shukla, 2010) and Grande Naine (Patel & Shukla, 2010) from different banana growing regions. After conducting studies on bacterial corm and rhizome rot in ten different banana varieties in Papua New

**Table 2:** Growth performance of the rescued accessions of NR during establishment (60 days after planting, DAP) and post-establishment (120 DAP) phases.

DAP	Plant Height (cm)		Collar diameter (mm)		Number of suckers		Number of leaves		LYOL (cm)		WYOL (cm)	
	60	120	60	120	60	120	60	120	60	120	60	120
Mean	60.4	79.1	63.0	76.6	2.0	0.7	9.2	8.8	68.6	83.9	29.0	30.8
t-test	**		**		**		ns		*		ns	

ns: non-significant, \*: significant at 5% and \*\*: at 1% level of significance using paired t test

**Table 3:** Growth performance of the rescued accessions of EB during establishment (60 DAP) and post-establishment (120 DAP) phases.

DAP	Plant Height (cm)		Collar diameter (mm)		Number of suckers		Number of leaves		LYOL (cm)		WYOL (cm)	
	60	120	60	120	60	120	60	120	60	120	60	120
Mean	44.0	87.1	41.5	87.2	0.9	1.0	8.8	10.4	53.1	92.3	21.8	35.7
t-test	**		**		ns		**		**		**	

ns: non-significant, \*: significant at 5% and \*\*: at 1% level of significance using paired t test

2a



2b

**Fig. 2:** View of a recently planted field of rescued suckers (a); close up view of a newly planted sucker (b).

3a



3b

**Fig. 3:** View of a field of successfully established NR (a) and EB (b) plantlets.

Guinea, Tomlinson *et al.* (1987) suggested that the genetic makeup of a plant plays a vital role in deciding its susceptibility to a pathogen. Varied degrees of incidence and disease severity have been recorded in different varieties in previously described reports e.g. 37 % in Dwarf Cavendish, 20–30 % in Grande Naine and 10–15 % in Gros Michel. The variable resistance reactions exhibited by different varieties and accessions in the present study may therefore be due to genetic differences between the plants.

Better vegetative growth improves the reproductive growth in all the plants, and banana is no exception. Positive correlation between yield parameters and vegetative growth parameters, mainly plant height and girth has been reported in Yangambi km 5 and Datil cultivars in Costa Rica (Vargas & Sandoval, 2005). Also, the plant height and collar diameter were found to be highly correlated with the days for flowering in trials in Nigeria (Blomme *et al.*, 2006).

The plants which get properly established in the field can absorb nutrients supplied to them effectively and thus can carry out cell division more efficiently. During transplanting, most of the plants had a few or no roots and thus it was difficult for the plants to absorb any nutrients from the soil. As opined by Blomme *et al.* (2006), if optimum growing condition along with proper supply of water, nutrients and sunlight is provided, bananas can grow very well even with a less developed root system. During our experiment, all these conditions were provided at optimum level. Furthermore, mono ammonium phosphate which contains about 61 percent of P<sub>2</sub>O<sub>5</sub> (P is an element actively involved in the root formation in plants) was applied through foliar sprays. These practices might have boosted plant growth in post establishment phase.

Number of suckers produced by the plant depends on rhizome size, number of dormant buds present on it, and amount of nutrients stored in it. It is also a parameter that reflects positive correlation with induction of flowering (Blomme *et al.*, 2006). During planting, the plantlets hardly had any stored tissues as only few centimeters of healthy tissue remained intact. Many plants weighed just 200–400 g (data not shown) as compared to the standard planting material of 1.5–2.0 kg each. As the plants started establishing, the recovery of the lost tissues improved and thus suckers were produced in some accessions. However, the numbers of suckers was low in both varieties 120 DAP owing to the desuckering operation performed in the plantation at monthly intervals. The case was similar with number of leaves. All the old, diseased and dried leaves were removed to maintain sanitation in the field and thus the parameter did not differ significantly in two phases.

## 5 Conclusion

Erwinia Rot, though considered as a minor disease, can cause considerable damage if not managed properly. Diversity existed in the accessions as different lines showed varied levels of infection. The rescue method described was highly effective for rescuing suckers heavily infected with the pathogen. A proper package of rescue techniques and appropriate supply of water, nutrients and growth conditions could result in improved establishment and subsequent growth of rescued plants.

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