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# A method to use very small size potato (Solanum tuberosum L.) tubers as seed

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Potato, being a vegetatively propagated crop, tubers of 30-60 g size is used as seed. Whereas, < 20 g size potato tubers are left in the soil itself after harvesting, as they are not suitable either for consumption or seed purpose. Seed pelleting technique is used to increase the tuber size and provide additional nutrient support. Two cultivars of potato (*Solanum tuberosum* L.) with five different treatments using left over under sized tubers (having radius of around 3.98 to 5.57 mm) were used for this experiment. During storage, weight loss was minimum in *Acacia* treatment (8.33%) and sprouting was not affected by *Acacia* and salicylic acid treatments. At the end of storage, phenol was high in *Acacia* treated tubers. These tubers were planted in the field. The time taken for 50% germination was similar in control (22.33 days), *Acacia* (22.33 days) and salicylic acid (22.67 days) treatments. Varietal differences were observed with the type of chemical used for pelleting. In general the yield obtained with *Acacia* leaf powder pelleting was more in both the cultivars.

Key words: Seed potato, pelleting, Acacia nilotica and sprout.

# INTRODUCTION

Traditionally, potato (*Solanum tuberosum* L.) is being propagated vegetatively by all over the world. The proportion of cost involved towards seed tubers in potato seed production is alarming to an extent of 40% (Sarjeet Singh and Naik, 1993) to 70% (Almekinders et al., 1996). In India, the seed rate is 2.5 t ha<sup>-1</sup> (Jagpal Singh, 1993) because tubers are sold in kilograms not by numbers. This prejudices to find a suitable alternate method to reduce the cost of seed material. Few available methods are using true potato seed (TPS), cut potatoes, sprouts, sprout cuts, single node cuts, micro and mini tubers. TPS technology is added with many advantages like cost

effective, easy transport, less disease incidence etc but the adoption by farmers is less (Chilver et al., 2005; Rowell et al., 1986; Vander Zaag et al., 1989; Benz et al, 1995; Sikka et al., 1994; Pangaribuan, 1994; Adhikari, 2005; Gupta et al., 2004) particularly where land availability is more for potato cultivation. The other technique, using "cut potato" but, poor performance is reported by Nielson et al. (1989); Rykbost and Lockeil (1999) and chances of virus multiplication, which may in turn lead to economic loss to the grower. Potato is infected with nearly 40 species of viruses (Valkonen, 2007). Potato is a vulnerable crop for many viruses;

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many of them are transmitted mechanically except few like PLRV (Mike Mayo et al., 2000). Cuts transmit more viruses, hence adaption is difficult. The other options are microtubers and minitubers. Microtubers are small potato tubers produced *in vitro* by plant lets in controlled condition inside the test tube or jar. Long term storage and its performance in the field when directly planted is questionable (Paolo Ranalli, 2007). Minitubers are the produce of *in vitro* plantlets in the field. These tubers are also small tubers range from 0.1 to 10 g or more (Struik, 2007). Here, the cost per tuber is much higher than the conventional one due to its procedural cost.

Making use of conventionally produced, small size left over tubers which are of no use otherwise is another way, if the size of the tuber is increased to acceptable planting tuber size and addition of nutrients is made to give a proper kick start to plant growth under field condition. Seed pelleting, is a technique for coating seed used to increase the size of very small horticultural seeds which provides improved planting features and is a potential alternative for improving sowing efficiency. Pelleting allows the seeds not only to gain both weight and volume but also standardizes seed size. The materials used for coating seeds are classified into two groups: binder materials and bulking or coating materials with or without micronutrients or growth regulators for the invigoration of the planting material. Furthermore, the pellet's external surface is smooth and not deformed, facilitating its planting by seed drills if it is used for mechanical planting. Pelleting is mainly used for seeds which have a high market value, such as tobacco, eucalyptus, some vegetables and ornamental plants, but is uncommon for smaller seeds, such as grasses, which have a lower market value, and high volume high value crops like potato. This has been successfully practiced in many crops like chilli, groundnut, soybean etc., but, information on their effect on seed potato is scanty. Keeping the above facts in mind, a trial was initiated in two cultivars of potato (Kufri Swarna and Kufri Jyoti) during the year 2009 and 2010. Pelleting was chosen because of its advantage over priming as reported by Murata et al., (2008) in groundnut. To our knowledge, this is the first attempt which indicates the possibility of pelleting the potato tubers. Acacia nilotica leaf powder is used as a base coating material for pelleting. The use of Acacia leaf powder as pelleting agent was reported by Nargis et al (1999). Acacia species are used to treat diseases of livestock as reported by Dafallah and Al-Mustapha (1996), Shah et al. (1997), Amos et al. (1999), Gilani et al. (1999) and Hussein et al. (2000). Hence, we attempted to use the Acacia in our study.

#### MATERIALS AND METHODS

#### Experimental site and material details

The trials were conducted at Central Potato Research Station,

Muthorai, Tamil Nadu, India located at an elevation of 2140 m above MSL at 11° 24' North latitude and 74° 4' East longitude. The normal rainfall of the location is 1300 mm, received in 85 rainy days and it is well distributed during both South West and North East monsoons. The mean maximum and minimum temperatures of the region range between 15 and 23°C and 6 and 13°C, respectively. The climate of the region is moist sub humid type (Manorama K, 2004.). The soil type of experimental site is sandy clay loam with a pH range of 4.7 to 5.0, 0.1 dSm<sup>-1</sup> EC and high in available nitrogen, low in available phosphorus and high in available potassium. The very small undersize seed tubers, left over from foundation seed multiplication plots of cultivars Kufri Swarna and Kufri Jyoti were collected manually at Central Potato Research Station, Muthorai (PO), The Nilgiris, India constituted the study material for the present investigation. The collected tubers were stored for 15days at room temperature in an average temperature of 20°C with a relative humidity of 85% for curing. The average size of tubers was 3.98 to 5.57 mm radius with 13.78 g of weight. The radius was measured with a help of vernier calipers.

The yield potential of Kufri Swarna and Kufri Jyoti is 25 t ha<sup>-1</sup>. The experiment was conducted with tubers collected from summer harvest of 2009 and 2010 with five treatments. Ten tubers were used for each treatment for field experiment and five tubers for further analysis. The treatments were T1- Dried Leaf powder of *Acacia nilotica* (AN) alone, T2- Dried Leaf powder of *Acacia nilotica* + Calcium Sulphate (CS), T3 - Dried Leaf powder of *Acacia nilotica* + Salicylic acid(SA), T4 - Dried Leaf powder of *Acacia nilotica* + Triiodo benzoic acid (TIBA) and T5 - No pelleting (Control).

#### Pelleting material

The matured green leaves were collected from the *Acacia nilotica* plants from nearby Mettupalayam forest and shade dried. Well dried leaves were kept under oven at 50°C for 2 hours and powdered with the help of a grinder. Fine powder was collected through 300 mm sieve trays. Maida, a finely milled and refined flour of wheat using a fine mesh of 600 meshes per square inch was used as adhesive. Calcium sulphate (Brand:CDH), Salicylic acid (Brand :Merck) and TIBA (Brand: Merck) were purchased from a commercial supplier.

#### Pelleting methodology

Bulking (or coating) material used was *Acacia nilotica* leaf powder with respective chemicals and 2% Mancozeb (Indofil company) as antifungal as per treatments. The binder was prepared by Maida flour of 20 g into 80 ml of distilled water until a smooth paste is achieved by light heating and brought to the normal temperature before use. The binder should not be too thick or too drippy. The well mixed bulking material (*Acacia nilotica* leaf powder) was taken in a separate bowl and tuber placed into it; then the binder was added slowly in to the bowl containing tubers by gentle rotation. Repeated coating was performed by intermittent shade drying till the desirable size. An average of 13.79 g of bulking material along with binder was used for a tuber. The tubers were dried for an hour on the paper towel before stored.

Pelleted tubers were stored at normal storage with a temperature range of 5.7 to 18.5°C and the RH range of 49.2 to 81.3% for 150 days. Observations were taken at 15 day intervals throughout the experiment. Sprouting rate, weight loss and length of sprouts were recorded.

**Sprouting rate:** At the end of every 15 days of storage, the numbers of sprouted tubers were counted. Sprouted tuber percentage was worked out in each treatment and expressed in percentage.

# Sprouted tuber number at the end of 15 days of storage

Sprouted tubers (%) =

# Sprouted tuber number at the beginning of 15 days of storage

**Tuber weight loss:** Tuber weights (10 tubers) were recorded prior to storage, and after every 15 days of storage. Weight loss was expressed as percentage: [(tuber weight prior to storage - tuber weight after every 15 of storage) / tuber weight prior to storage] x 100.

**Length of the sprout:** Tuber sprout length (10 tubers) was measured at the end of storage period in each treatment with the help of vernier caliper and expressed in millimeter.

At the end of storage, tubers were taken for field experiment. Randomised block design Design (RBD) with three replications. One row of 10 tubers each, with a spacing of 40 x 20 cm were planted. Farmyard manure and fertilizers were applied as per recommendation. One per cent urea spray was given at  $45^{\text{th}}$  and  $60^{\text{th}}$  days after planting on the foliage.

After maturity, tuber yield and number was recorded in three different grades (<30, 30-60 and >60 g) by grading from the row and was expressed in kg per 10 plants and number per 10 plants.

**Bio chemical analysis:** Reducing sugars was estimated by using the method of Nelson (1944), Total free amino acids by Roe et al., (1990) and Phenols by Sadasivam and Manickam (1992). For this purpose, three tubers were selected randomly and the analysis was carried out.

#### Field potential of pelleted seed tubers

#### Tuber yield in different grades

Tuber yield was recorded in three different grades (<30 g (small size), 30-60 g (seed size) and >60 g (large size)) by manual grading and the weight was expressed in kilogram.

#### Tuber number in different grades

Tuber number was recorded in three grades (<30 g (small size), 30-60g (seed size) and >60g (large size)) and it was expressed in numbers.

#### Statistical analysis

Data were analyzed using Analysis of Variance with mean separation by LSD Test using WASP 2, a web based software developed by ICAR research complex, Goa, India. Percentage data was arc-sin transformed before analysis.

# RESULTS

Weight loss (%): Tuber weight loss was recorded from the day of storage till 150 days at 15 days interval. In control, out of the two varieties tried, the per cent weight loss of stored tubers was more in K. Swarna (16.02%) than that of K. Jyoti (14.92%). In K. Swarna, weight loss started after 15 days of storage in CS, TIBA and control whereas it started at 45 days in SA and at 50 days after storage in AN treatments. The same trend was observed in K. Jyoti cultivar except in AN treatment where weight loss has started after 75 days of storage (Figure 1b and 1e). Loss of weight in storage is expected to reduce the vigour of the tubers when planted in main field. The delay in weight loss was observed in SA and AN treatments, and the delay was more spectacular in AN treatment in both the varieties. Even at 150 days after storage the weight loss in AN treatment was nearly 50% only in K. Swarna and it was up to 65% only in K. Jyoti when compared with control. This indicates that AN was effective in reducing the tuber weight loss in storage.

- x 100

**Sprouting (%):** All Pelleted tubers sprouted well after 150 days of storage in both the cultivars. In control sprouting started at 45 days in both the varieties. But the AN treated tubers started sprouting only after 90 days in K. Jyoti and at 75 days in K. Swarna (Figure 1a). All the tubers sprouted within 120 days in control in both the varieties. Hundred per cent sprouting of tubers was recorded at 120 days in TIBA in both the varieties. In K Jyoti CS treatment recorded cent per cent sprouting at 150 days and in K Swarna at 135 days. (Figure 1d). However, in CS treatment sprouting started at 15 days and it progressed slowly. The rate of sprouting could be controlled greatly by AN treatment in both the varieties and more effectively in K Jyoti.

**Sprouting length (mm):** Pelleting significantly influenced the length of sprouts during storage of potatoes in both the cultivars. The highest sprout length of 56.29 mm was recorded at 150 days in K. Swarna in CS treatment and it was 47.30 mm in K. Jyoti at 150 days under the same treatment. Minimal length of longest sprout was recorded in AN (8.02, 9.24 mm) followed by SA (9.56, 12.63 mm) in K. Swarna and K. Jyoti, respectively at 150 days achieved in K. Swarna followed by K. Jyoti (47.30) (Figure 1c and 1f). In K. Swarna the per cent reduction in longest sprout length has been to the tune of 17% in comparison with the control and it was 26% in K. Jyoti in AN treated tubers at 150 days. Among the different pelleting treatments AN has proved to be more effective in reducing the sprout length in both the varieties.

**Biochemical analysis:** Higher content of reducing sugars was recorded in SA (294.89) treatment in K. Swarna and it is 320.50 in K. Jyoti under AN treatment. Control recorded the lowest values for both the varieties. Higher total free amino acids were recorded in CS treatment in both the varieties and the lowest values were observed in control. Higher phenol content was recorded in AN treatment for both the varieties (Figures 2 and 3) and the lowest were recorded in control.

*Field performance*: The time taken for 50% germination was similar in Control (22.33 days), AN (22.33 days) and

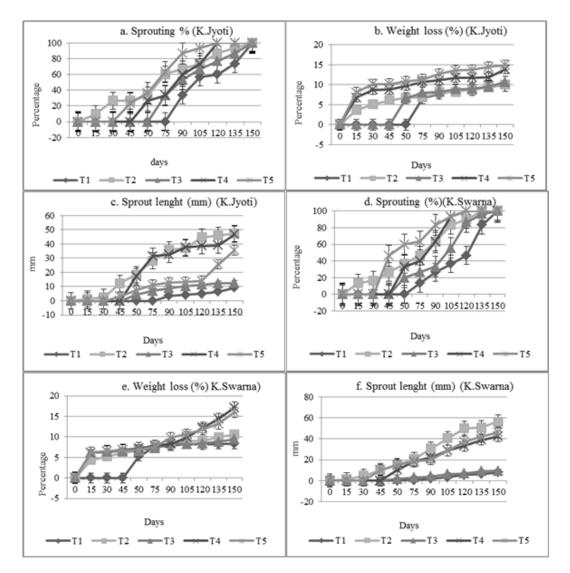
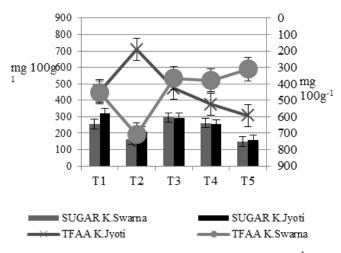


Figure 1. Effect of pelleting on sprouting (%), weight loss (%) and sprout length (mm) in K. Jyoti and K. Swarna.



**Figure 2.** Efffect of pelleting on free sugars (mg 100  $g^{-1}$ ) and total free amino acid (TFAA) after storage.

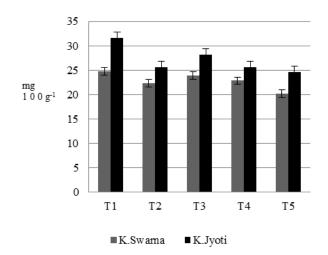


Figure 3. Efffect of pelleting on phenol (mg 100  $g^{-1}$ ) after storage.

Cultivar	Treatment (T)	Numbers of tubers (numbers 10 plants <sup>-1</sup> )				Weight of tuber (Kg 10 plants⁻¹)			
		<25 g	25-75g	>75g	Total	<25g	25-75g	>75g	Total
V1	AN	16	163	7	186	0.30	0.98	0.90	2.18
	CS	9	125	3	137	0.17	0.84	0.25	1.25
	SA	12	156	5	173	0.16	0.98	0.45	1.59
	TIBA	14	155	4	173	0.21	1.06	0.38	1.65
	Control	9	115	1	125	0.19	0.69	0.10	0.98
	Mean	12	143	4	159	0.20	0.91	0.42	1.53
V2	AN	11	211	7	229	0.17	0.91	0.93	2.01
	CS	10	200	2	212	0.14	1.10	0.20	1.44
	SA	11	204	5	220	0.22	1.24	0.45	1.91
	TIBA	11	195	5	211	0.17	1.16	0.54	1.87
	Control	4	122	0	126	0.09	0.82	0.00	0.90
	Mean	9	186	4	200	0.16	1.05	0.42	1.62
		CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)	CD(P <u>&lt;</u> 0.05)
v		1.08	9.84	NS	20.93	0.02	0.05	NS	0.95
t		1.71	15.56	0.42	40.25	0.03	0.09	0.05	1.50
v xt		2.42	22.01	0.60	NS	0.05	0.12	0.07	NS

Table 1. Field performance of pelleted potato seed tubers.

V1 = K. Swarna V2 = K. Jyoti NS = Non-Significant.

SA (22.67 days) treatments. But in due course of time, all the treatments achieved cent percent germination. Significant differences were noticed in field performance of different pelleting treatments in both the varieties tested. In both the varieties, seed sized tuber number (163, 211) as well as yield (0.98, 0.91) and total tuber number (186, 229) and yield (2.18, 2.01) (for K Swarna and K. Jyoti, respectively) were more in AN treatment. The increase in tuber number (49%) was highly significant in K. Swarna which is a shy bearing with reference to tuber number. In terms of yield, the increase is more in K. Swarna as it produced almost double the yield than the control (Table 1).

## DISCUSSION

The rationale for seed pelleting is to make the seed perfect for planting by increasing the size as well as making the nutrition sufficient enough to give growth in the field. Selection of binder materials and bulking or coating materials is important for pelleting. Pellet forming is an artistic method combined with science.

In our experiment, vigor enhancement by way of early sprouting was noticed in SA treatments in both the cultivars by nearly 30 days over other treatments in both the cultivars. Salicylic acid (SA) is known for its defense reactions induced endogenously (Gaffney et al., 1993; Clarke et al., 2000) but effect on germination is not well defined because in some crops it inhibited the germination namely, maize (Guan and Scandalios, 1995) and caused vigour enhancement in wheat (Shakirova et al., 2003) and pea (McCue et al., 2000). Vigor enhancement by the incorporation of growth regulators in pelleting might be due to increased cell division and hormonal balancing system which caused an increase in sprout growth. Many basic works have been done in Arabdiobsis to understand the role of SA on seed vigour and found that SA plays a major role from seed storage protein mobilization to protein translation which in turn increase the seed vigour. Finally, the proteomic data revealed a close interplay between abscisic (ABA) signaling and SA elicitation of seed vigor.

Abscisic acid (ABA) reduction is believed to be important for dormancy breaking which in turn leads to sprout initiation in potato. Still, this phenomenon is controversial (Ume Sonnewald, 2001) as decline of ABA did not correlate with the sprouting behavior of the tubers, ruling out the possibility that the decline of ABA content below a certain threshold level is responsible for the break of dormancy (Biemelt et al., 2000). Although, in our experiment, early sprouting has indicated that SA as growth regulator might have played some role in altering the growth promotor (GA and cytokines) and inhibition (ABA and ethylene) ratio to promote the sprouting.

In general, calcium is amended as a nutrient in the soil to enhance the quality of potato because of its direct correlation with post-harvest quality and to enhance the drought tolerance in potato cultivation. Calcium is important for the growth of sprout in potato and to have the apical

 Table 2. Phytochemical Components of A. nilotica ethanolic leaf extract

Secondary metabolites	Ethanolic leaf extract				
Saponon	+				
Saponin glycosides	+				
Volatile oil	+				
Hydrolysable tannin	+				
Steroids	0				
Triterpenoid	+				
Tannin	+				
Flavonoids	+				
Phenol	+				
Alkaloid	+				

+ = Presence, 0 = absence (Solomon and Shittu, 2010).

dominance, which arrests the lateral branches. Dark storage creates calcium deficiency and causes sub-apical necrosis followed by death (Dyson and Digby, 1975(a)). In pelleting, the tubers are covered by pelleting material and a dark condition is created for the tuber. Calcium Sulphate was added in one of the treatments to understand and to avoid the formation of sub-apical necrosis.

Calcium sulphate pelleted tubers were able to sprout within 60 days after storage in both the cultivars when compared with control (45 days) showing its ability to overcome the sub-apical necrosis. In all the treatments sub-apical necrosis was not noticed. The sprouting was delayed by 15 days in CS when compared with control. This shows that some physiological activities either dormancy breaking or sprout initiation was modified. Arrest of physiological ageing through calcium application is tested by Dyson and Digby (1975b). Further, Booth (1963) showed that lateral branching is due to poor interaction between auxin and gibberellin.

Tri-iodo-benzoic acid (TIBA) was added to the experiment to understand the role of an inhibitor in the pelleting process. TIBA was identified as auxin inhibitor by Kuse (1953) when he found that auxin moving from the leaf did not pass the point where the TIBA was applied in a band of lanolin paste on the petiole. In this paper, these deductions have been tested by direct methods. Further this was confirmed by Muller (1999) in wild type peas. Ana Rincon et al. (2001) hypothesized that TIBA prevented fungal IAA transport towards the root Laccaria bicolor. In our experiment, tubers pelleted with TIBA took 60-75 days for sprouting when it is only 45 days in control. Sprout growth was inhibited by TIBA for 15 to 30 days. This might be due to the alteration in the auxins level. Though auxins did not play a direct role in the sprouting but played a major role in the dormancy breaking mechanism which is a pre request for the sprout initiation.

The sprouting percent was affected by *Acacia* leaf powder pelleting. It took 90 days to start sprouting which

is 45 days later than control. The highest total extractable phenolics and total extractable tannin values were recorded in *A. nilotica* (168.36 and 176.15 mg  $g^{-1}$  DM, respectively) (Mtui Dorah, 2008). Pelleted with acacia leaf powder delayed germination might be due to the less accessibility to oxygen by the sprouts.

Many phenolic compounds are reported in the potato tubers (Uppal and Verma, 1982; Ghanekar et al., 1984). These compounds are the substrates of polyphenoloxidase enzyme and are involved in enzymatic browning of peeled potatoes (Mapson et al., 1963), in resistance of potatoes to diseases (Tripathi and Verma, 1975) and further reported that a decrease in the content of total phenols in the peels and pulp of two varieties of potatoes stored under ambient conditions. In the present investigation, the there is significant change in the phenol content among the treatments and between varieties. The chemistry of A. nilotica leaf extracts has shown the presence of phenol (Table 2) which played a role when it is coated over the tubers especially giving good health in the field by avoiding the diseases. Though there is a significant difference among the treatments in the free sugars and total free amino acids, a definite correlation could not be arrived.

The early achievement of 50% germination in the field by control (without coating) which is one of the vigour indicating factors may be due to non-hindrance by coating. Reduced emergence due to pelleting is possible when clay is applied over sugar beet seeds (Durant and Loads, 1986) but macronutrient solution immersed seeds vielded with more in cereals (Woomer et al., 2003) and nutrient pelleted seeds accelerated growth and development (Konstantinov, 1983) which has not happened in our experiment with potato. There is a significant difference between A. nilotica pelleting alone with that of added nutrients treatments. The performance of pelleted seed with other growth regulators might have affected the chemical transformation in the plant system. Further, Razzaque et al. (2004) reported that the application of TIBA had reduced the height of tomato plants. The increase in the tuber number and the total yield by A. nilotica coating is due to its antimicrobial activity, disease resistance is due to high phenol content and nonhindrance with other nutrients during growth period which facilitated the good source to sink movement during the bulking stage, a crucial period in potato growth.

# Conclusion

Potato seed tuber which acts as a mother, supplies the food to growing sprout till it starts photosynthesis. This feeding results in vigorous and rapid crop growth during early growth stages, which probably leads to higher tuber yields. The vigorous sprout growth is due to the availability of external nutrients applied to the acacia leaf powder and suppression of tannins and phenols available in the acacia leaf powder. Acacia leaf powder alone is having the potential to prolong the shelf life of very small left over tubers in the field and to give little lesser than the normal potato yield when it is planted in the field without much extra care. Hence, very small potato seeds which or otherwise waste in the field can be used as potato seed by modifying its size by pelleting where the seed cost is very high depending upon the labour availability.

## **Conflict of interest**

The authors have not declared any conflict of interest.

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