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# Potato Journal

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The Indian Potato Association**

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# DIFFERENTIAL BEHAVIOR TO DROUGHT OF TWO ELITE POTATO CULTIVARS GROWN IN SOUTHEASTERN PAMPEAN REGION OF ARGENTINA

Martin Tagliotti<sup>1\*</sup>, Sergio Giuliano<sup>2</sup>, Marino Puricelli<sup>1</sup>, Andrés Zambelli<sup>2</sup> and Maria Cecilia Bedogni<sup>1</sup>

**ABSTRACT:** Potatoes production requires large inputs of water for which development of drought tolerant cultivars is particularly relevant to ensure or even increase its productivity. Two elite cultivars, Newen INTA and Spunta, were evaluated in the field under contrasting water regimes. After drought treatment, the yield of marketable tubers decreased 53% in Spunta and 22% in Newen INTA while the number of marketable tubers decreased 32% in both cultivars. Yield of tuber > 90 mm only decreased in Spunta (62.81%,  $p < 0.05$ ). Newen INTA showed a greater reduction in the shoot and root dry biomass than Spunta, while the root/shoot ratio was higher in Newen INTA. Proline content increased 61% in both cultivars under drought treatment. Drought tolerance indices (GMP and DTI) were higher in Newen INTA, and the drought susceptibility index was higher in Spunta. Proline content was correlated with root dry biomass and the marketable tuber number in both cultivars. Dry root biomass correlated with the yield of marketable tubers and the number of marketable tubers. In drought conditions, Newen INTA showed greater tolerance to drought. Therefore, Newen INTA emerges as a useful genotype to contribute to increasing crop productivity in areas subject to water stress.

**KEYWORDS:** drought tolerance; Spunta; Newen INTA; drought stress indices; proline content

## INTRODUCTION

Potato (*Solanum tuberosum* ssp. *tuberosum* L.) is a crop with high to moderate sensitivity to drought (Hijmans, 2003; Monneveux *et al.*, 2013) showing limited yield in arid and semi-arid regions. In 2019, potato world production was around 380 million tons, on a planted area of 19.3 million hectares (FAOSTAT, 2023). Its susceptibility to water stress makes that a regular supply of water is necessary to achieve acceptable yield and quality (Boguszewska-Mańkowska *et al.*, 2020; Ierna *et al.*, 2011; Iwama, 2008; Levy *et al.*, 2013; Wang *et al.*, 2006) for which the development of drought-tolerant cultivars is a highly valued objective to achieve an efficient and sustainable production. Among the main biochemical mechanisms involved

in drought tolerance, the accumulation of osmotic compounds such as free proline was described to play a relevant role (Evers *et al.*, 2010; Rai *et al.*, 2011). For instance, the variation in proline content described in plants with contrasting drought tolerance was associated with a differential osmoregulation capacity (Schafleitner *et al.*, 2007; Szabados and Savouré, 2010). Tagliotti *et al.* (2020) reported a negative correlation between yield and proline content working with a very diverse potato panel, showing that the response to water stress was also influenced by the genetic background (Hassanpanah, 2010; Sprenger *et al.*, 2018).

Potato is the most consumed vegetable in Argentina, with 40.8 kg per person, concentrating 55% of the national production

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in the southeastern (SE) Pampean region where the crop is mostly grown under irrigation (MAGYP, 2021). *S. tuberosum* ssp. *tuberosum* cv. Spunta is the most produced cultivar in Argentina, representing 90% of the local consumption (Huarte and Capezio, 2013; MAGYP, 2021). Newen INTA is a promising cultivar developed by INTA (*Instituto Nacional de Tecnología Agropecuaria*, Argentina) for fresh consumption and food industry (Variety Register Number 12126; <https://gestion.inase.gob.ar/consultaGestion/gestiones>). Under water stress conditions Spunta was described as sensitive or moderately sensitive (depending on growing conditions) when compared with Newen INTA cultivar (Bedogni *et al.*, 2018; Lerna and Mauromicale, 2006). To confirm this observation, drought responses of Spunta and Newen INTA were compared in the field under differential water regimes focusing on yield, tuber quality and the proline-mediated osmoregulatory response.

## MATERIALS AND METHODS

### Experimental design and measurement of soil and climate parameters

Field trials were conducted during 2019-2020 growing seasons in Balcarce, Argentina (37° 45'32" S, 58° 17' W, 100 m.a.s.l.). Spunta and Newen INTA cultivars were planted in October 2019 and harvested in March 2020 (123 days of crop cycle). The average relative humidity was  $72.4 \pm 9.3\%$ , and the mean temperature was  $19.23 \pm 3.38$  °C. The minimum and maximum temperatures were  $12.2 \pm 4.3$  °C and  $27.2 \pm 4.6$  °C, respectively. Cumulative solar radiation ranged from a minimum of  $6.9$  W m<sup>-2</sup> in November and March and a maximum of  $7.8$  W m<sup>-2</sup> in January. The soil was a typical Argiudoll (Mar del Plata soil serie; Soil Survey Staff, 2006) with 5% organic matter and a pH of 5.7 (<https://zenodo.org/records/6353509>;

8/10/2023). Five virus-free tuber seeds per meter were planted in 5 m rows spaced by 0.85 m. The experimental design was a complete randomized block with four replicates and four rows per block. At planting time, plots were supplemented with 200 kg ha<sup>-1</sup> of diammonium phosphate. To minimize biotic stresses, fungicides and insecticides were sprayed (Infinito™ [Fluopicolide 6.25% + Propamocarb hydrochloride 62.5%], Bayer CropScience; Poliram DF™ [Metiram 70%], BASF Chemistry) following the supplier's recommendations. Weeds were manually controlled.

Field capacity (FC, %), and permanent wilting point (PWP, %) and bulk density were measured to evaluate available water in the soil. These values were used as input variables to estimate the soil water holding capacity (SWHC) and establishing the irrigation protocol. Therefore, at planting time, soil water conditions at depths between 0 and 20 cm, SWHC was 33.7 mm, between 20 to 40 43.5 mm. The irrigation protocol was divided into "irrigated treatment" and "drought treatment". Irrigation treatment was applied by sprinkling when the 60% of the SWHC was reached and consisted in twenty doses of water of average 16.1 mm each added throughout across the crop cycle. In drought treatment, plants grown with no irrigation (dry-farming conditions) during the whole crop cycle. The total water supplied under irrigation was 571.0 mm and under drought treatment 248.9 mm. The daily water balance at 40 cm depth of soil was calculated according to:

$$SWC_r = SWC_i + NP + NI - RET * Kc$$

Where  $SWC_r$  is the soil water content on day  $r$ ,  $SWC_i$  is the soil water content on the previous day  $i$ . NP is the net precipitation, NI is the net irrigation and RET is the evapotranspiration reference, and Kc is the crop coefficient on day  $r$  (Allen *et al.*, 2006). The NP was calculated with a run-off coefficient of 0.1, and to calculate the NI an efficiency of 0.6 was assumed (Della Maggiora, 1996). The chart in Fig. 1 shows the

soil and climate parameters collected during the assay. SWC<sub>r</sub> values below the 60% SWHC were not recorded.

### Sampling and measurement of proline content

Proline content was measured at 46, 60, 75 and 90 days after planting in fully expanded leaves of the third level relative to the top of the plant. Each sample consisted of four plants harvested from the outer rows. Leaf tissues were collected between 9:00 a.m. and 10:00 a.m. taking 0.1 g fresh tissue which was immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until processing following Bates' protocol (Bates *et al.*, 1973) modified by Carillo and Gibon (2011). The sap of each frozen leaf tissue was collected with an electric roller press (Sew-Eurodrive D752) and diluted 20-times with a 70:30 ethanol/water mixture (v/v) (Hummel *et al.*, 2010). A 1:1 mixture of extraction homogenate and reaction mix (ninhydrin 1% [w/v] in acetic acid 60% [v/v] and ethanol 20% [v/v]) was poured into 2-ml Eppendorf tubes and heated at  $95^{\circ}\text{C}$  in a water bath for 20 min. After cooling to room temperature, each tube was spun down (1 min, 500g) and 100  $\mu\text{l}$  of supernatant was transferred to a polypropylene microplate to measure the absorbance at 520 nm. The proline content was quantified by a calibration curve and expressed in  $\mu\text{g}$  per g of fresh tissue (FW) (Mohamed *et al.*, 2010).

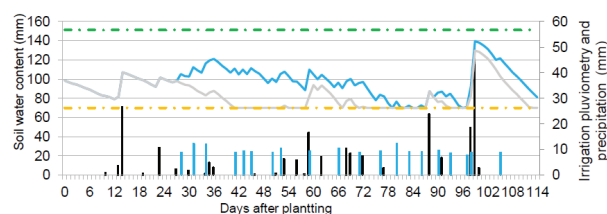


Fig.1. Soil water content and climate parameters measured during the assay: net precipitation (black columns); net irrigation (blue columns); water content of irrigated soil (solid blue line); water content of non-irrigated soil (solid grey line); water content in field capacity (dotted green line); water content at the permanent wilting point (dotted yellow line).

### Yield and tuber evaluation

For dry biomass measurements, fully mature plants were removed from inner rows keeping the surrounding soil within a radius of 20 cm and a depth of 40 cm (Lahlou and Ledent, 2005). Whole plants were immersed in water during 30–45 min to remove adhered soil and shoot and root parts (without tubers) were separated (Man-Hong *et al.*, 2020). Each fresh tissue was weighted (FW) and then oven-dried at  $60^{\circ}\text{C}$  until constant weight to obtain dry biomass values. Each replicate measured the dry biomass of roots, leaves, stems, and fruits (including dead material). The dry root/shoot biomass ratio (R/S) was calculated.

Total yield, marketable yield and tuber number were recorded. The tubers were classified by diameter (<50 mm - undersized; 50–75 mm - intermediate; 70–90 mm - upper intermediate; >90 mm - oversized), counted and weighted. Tubers without diseases or defects and larger than 50 mm were recorded to calculate marketable yield. A subset of the total harvest was used to measure the dry matter content, according to Cacace *et al.* (1994). The following drought tolerance indices were calculated: drought susceptibility index (DSI) (Fischer and Maurer, 1978), and geometric mean productivity (GMP) and drought tolerance index (DTI) (Fernandez, 1992):

$$\text{DSI} = [1 - (Y_s / Y_p)] / [1 - (\hat{Y}_s / \hat{Y}_p)]$$

$$\text{GMP} = (Y_p \times Y_s)^{0.5}$$

$$\text{DTI} = (Y_s \times Y_p) / (\hat{Y}_p)^2$$

Where  $Y_s$  is the marketable yield of a given genotype under drought,  $Y_p$  is the marketable yield of this genotype under irrigation treatment, and  $\hat{Y}_s$  and  $\hat{Y}_p$  are the mean marketable yields of all genotypes under drought, and irrigation treatments, respectively.

### Statistical analysis

To calculate the marketable yield, marketable tuber number and dry matter content, the fitted model was:

$$y_{ijp} = \mu + v_i + t_j + (vt)_{ij} + b_p + e_{ijp}$$

Where  $y_{ij}$  is the trait value,  $\mu$  is the overall mean,  $v_i$  is the fixed effect of the  $i$ th potato cultivar,  $t_j$  is the fixed effect of the  $j$ th treatment,  $(vt)_{ij}$  are the interaction effects between the previously fixed effects,  $b_p$  is the random effect of the  $p$ th block and  $e_{ijp}$  is the random error of the  $(ijp)$ th observation. This model assumes a distribution explained by  $b_p \sim N(0; \sigma_b^2)$  where  $\sigma_b^2$  is the variance of the random effect of the block and  $e_{ijp} \sim N(0; \sigma_e^2)$  where  $\sigma_e^2$  is the variance of the error.

For the shoot and root dry biomass, and proline content the fitted model was:

$$y_{ijsp} = \mu + v_i + t_j + m_s + (vt)_{ij} + (tm)_{js} + (vm)_{is} + (vtm)_{ijs} + b_p + e_{ijsp}$$

Where  $y_{ijsp}$  is the trait value, and  $\mu$ ,  $v_i$ ,  $t_j$ ,  $(vt)_{ij}$ , and  $b_p$  were defined above. The  $m_s$  is the fixed effect of the  $s$ th day of treatment,  $(tm)_{js}$ ,  $(vm)_{is}$ , and  $(vtm)_{ijs}$  are the interaction effects between the previously fixed effects and  $e_{ijsp}$  is the random error of the  $(ijsp)$ th of the  $s$ th repeated observation (Ashraf and Foolad, 2007). This model assumes distributions  $b_p \sim N(0; \sigma_b^2)$  and  $e_{ijsp} \sim N(0; \sigma_e^2)$  which were verified by graphical exploratory analysis of residuals and restricted likelihood ratio test. In the case of violation, alternative assumptions were proposed, evaluated, and checked. In each mixed linear model, ANOVA tests were performed for the main factors and their interactions. When statistically significant differences ( $p < 0.05$ ) were detected, a *post-hoc* pairwise comparison across each factor was performed by using the Tukey test.

The correlation between the traits studied was assessed using the Pearson correlation coefficient ( $r$ ) approach. All statistical analyses were performed using InfoStat software (Di Rienzo *et al.*, 2017) and R Project software (R Core Team, 2019).

## RESULTS AND DISCUSSION

### Yield performance and drought indices

Under drought treatment, Newen INTA presented a higher marketable yield (59.46 t ha<sup>-1</sup>) than Spunta (29.9 t ha<sup>-1</sup>). The percent reduction in marketable yield was 22.64% in Newen INTA and 53.39% in Spunta (Fig. 2a). The dry matter content of the tuber was not significantly affected under contrasting water regimes ( $p = 0.67$ ). The number of marketable tubers under drought decreased 31.07% in Newen INTA and 33.66% in Spunta (Fig. 2b).

The effect of drought treatment on yield was evaluated differentially according to the tuber size category (Table 1). In Newen INTA, drought treatment decreased yield respect to the irrigated for most size categories ( $p < 0.05$ ). Yield reduction of 29.51% was observed in tubers with diameter smaller than 50 mm,

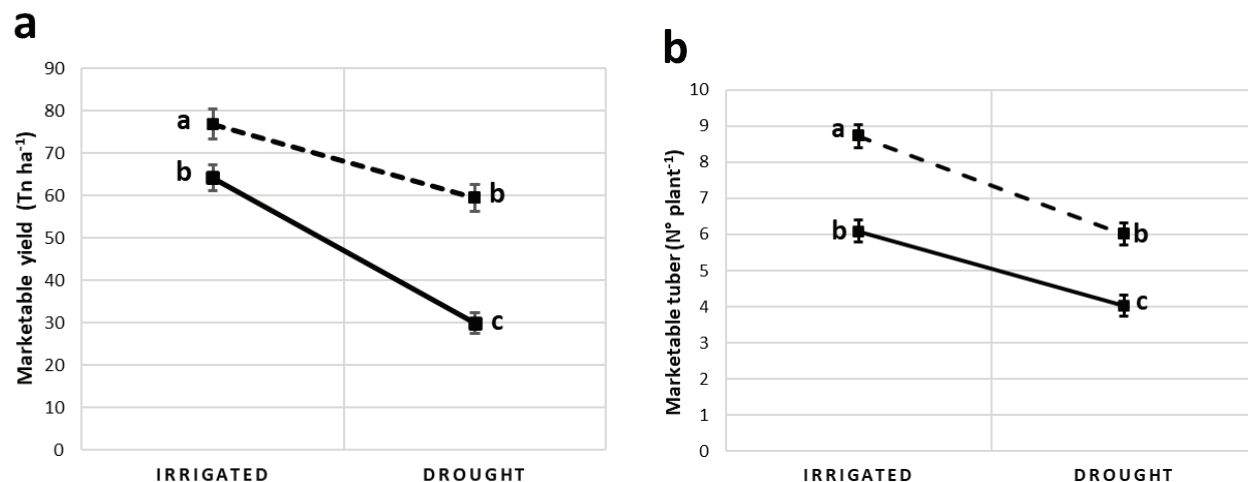


Fig.2. Mean yield performance of potato cultivars grown under irrigated and drought regimes. The marketable yield (A) and marketable tuber number (B) were measured at harvest. Newen INTA (dotted lines) and Spunta (solid lines). The thin bars represent the standard error. Values with the same letters indicate non-significant differences among mean values ( $P = 0.05$ ) according to a protected LSD test.

**Table 1.** Effect of contrasting water regimes on the yield of tuber size categories. Overall means  $\pm$  standard deviation. Means within the same tuber size category and with the same letters are not significantly different at  $P = 0.05$  according to a protected LSD test (ns: non-significant). YR: yield reduction.

Cultivar	Treatment	Yield of each tuber size category (t ha <sup>-1</sup> )			
		< 50 mm	50 - 75 mm	75 - 90 mm	> 90 mm
Newen INTA	Irrigated	6.10 $\pm$ 0.61 b	19.44 $\pm$ 1.7 a	25.43 $\pm$ 0.82 a	32.60 $\pm$ 2.74 b
	Drought	4.30 $\pm$ 0.56 a	7.32 $\pm$ 0.76 b	15.13 $\pm$ 1.01 b	36.42 $\pm$ 0.82 b
	YR	29.51%	62.34%	40.50%	ns
Spunta	Irrigated	6.63 $\pm$ 0.64 bc	2.86 $\pm$ 0.60 c	8.40 $\pm$ 1.16 c	52.90 $\pm$ 0.82 a
	Drought	7.94 $\pm$ 0.77 c	3.27 $\pm$ 0.61 c	6.78 $\pm$ 1.20 c	19.67 $\pm$ 0.82 c
	YR	ns	ns	ns	62.81%

62.34% in diameters between 50 - 75 mm, and 40.50% in diameters between 75 - 90 mm. Newen INTA tubers larger than 90 mm did not significantly modify their yield under drought. In Spunta, drought treatment significantly reduced the yield only for tubers with diameter larger than 90 mm, showing 62.81% reduction (Table 1).

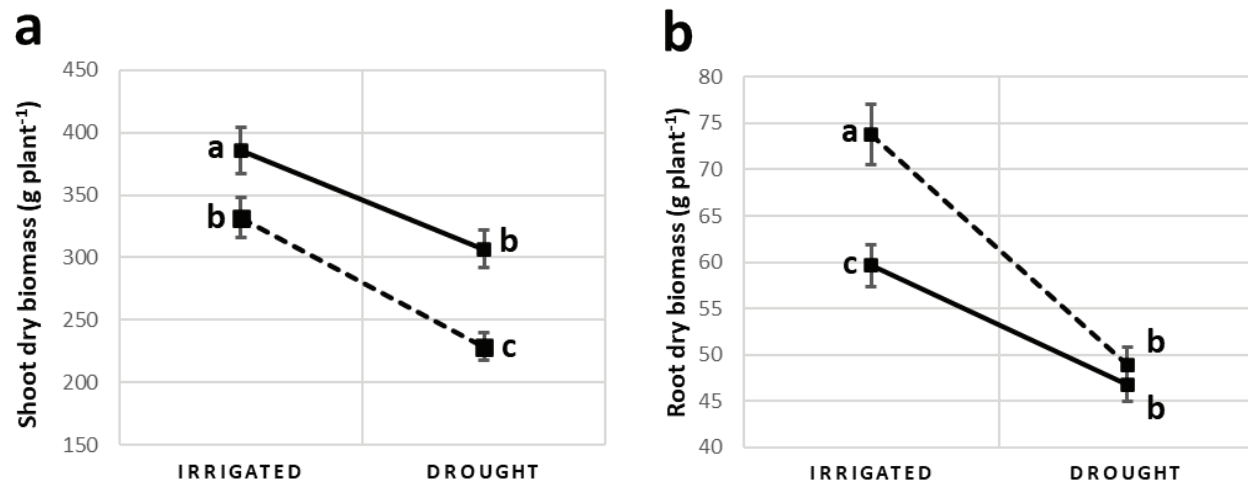
The stress intensity (SI) for both potato cultivars was 0.36. For such condition, three drought tolerance indices were evaluated by measuring the marketable yield. Thus, Newen INTA showed higher GMP and DTI, and lower DSI values when compared with Spunta (Table 2)

### Growth parameters

Under drought treatment shoot dry biomass decreased 31.09% in Newen INTA

**Table 2.** Drought tolerance indices of potato cultivars under contrasting water regimes. MY: marketable yield expressed as overall means  $\pm$  standard deviation.

Cultivar	Index	MY (t ha <sup>-1</sup> )
Newen INTA	GMP	67.52 $\pm$ 1.94
	DTI	0.91 $\pm$ 0.05
	DSI	0.37 $\pm$ 0.06
Spunta	GMP	43.56 $\pm$ 2.17
	DTI	0.38 $\pm$ 0.04
	DSI	0.84 $\pm$ 0.07



**Fig.3.** Growth parameters of potato cultivars under contrasting water regimes. *S. tuberosum* cv. Newen INTA (dotted lines) and cv. Spunta (solid lines) were cultivated under irrigation and drought regimes. The shoot dry biomass (a) and root dry biomass (b) were measured throughout the whole crop cycle. Thin bars represent the standard error. Means within the same trait with the same letters are not significantly different at  $P = 0.05$  according to a protected LSD test.

and 20.46% in Spunta (Fig. 3a). When dry root biomass was measured, drought treatment produced a decrease of 33.77% in Newen INTA and 21.58% in Spunta (Fig. 3b). The R/S ratio in Newen INTA was 0.20 and 0.15 in Spunta, regardless of the water regime; however, the difference was not significant.

### Proline content

The mean proline content between treatments was  $4.68 \pm 1.44 \mu\text{mol g}^{-1}$  FW in Newen INTA and  $8.89 \pm 1.42 \mu\text{mol g}^{-1}$  FW in Spunta (Fig. 4). The percentage of proline under drought increased 31.09% in Newen INTA and 20.46% in Spunta. The lowest proline content in Newen INTA was found 90 days after planting (Fig. 4).

### Joint analysis

Pearson correlation coefficients were calculated for marketable yield, tuber dry matter, proline content, shoot dry biomass, root dry biomass and marketable tuber number (Table 3). Marketable yield, root dry biomass and the number of marketable tubers correlated positively. Proline content, dry root biomass and the number of marketable tubers correlated negatively. The calculation of correlation matrix coefficients for each individual cultivar was consistent with the analysis grouping data from both cultivars.

Table 3. Pearson correlation coefficients (r) between the different parameters measured grouping data from both potato cultivars.

	TDM	PC	SDB	RDB	TN
MY	0.43ns	- 0.46ns	0.25ns	0.74**	0.91***
TDM		- 0.19ns	- 0.58*	0.33ns	0.53*
PC			-0.18ns	-0.57*	- 0.51*
SDB				0.45ns	0.21ns
RDB					0.87***

MY, marketable yield; TDM, tuber dry matter content; PC, proline content; SDB, shoot dry biomass; RDB, root dry biomass; TN, tuber number; ns: non-significant; \*\*\*significant at 0.001; \*\*significant at 0.01; \*significant at 0.05.

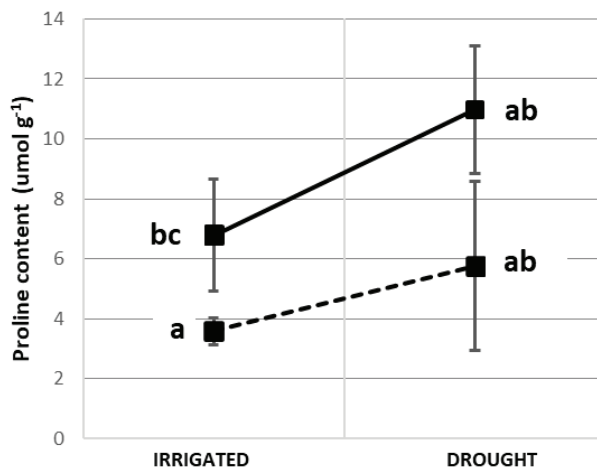


Fig.4. Proline content of potato cultivars under differential water regimes. Newen INTA (dotted lines) and Spunta (solid lines). Means represent the average proline content across the crop cycle. Thin bars represent the standard error. Means with the same letters are not significantly different at  $P = 0.05$  according to a protected LSD test.

Water deficit is the main factor affecting potato production and is responsible of significant reductions in yield, tuber size, and tuber number (Aliche *et al.*, 2019; Ierna and Mauromicale, 2006; Ierna *et al.*, 2011; Onder *et al.*, 2005). Therefore, identification of cultivars with tolerance to drought is highly valuable. Newen INTA and Spunta exhibited differential responses to water stress arising as a promising model to characterize the mechanism involved in. Spunta was confirmed as a drought susceptible cultivar, showing a greater reduction in marketable yield (53.39%). GMP and DTI were the parameters exhibiting the better ability to identify genotypes combining high yield potential and high yield under drought condition. Therefore, genotypes exhibiting higher GMP and DTI and lower DSI might be identified as drought-tolerant (Cabello *et al.*, 2013; Saravia *et al.*, 2016; Schafleitner *et al.*, 2007; Sharma *et al.*, 2011). Under the same stress intensity (0.36) Newen INTA presented higher GMP, DTI and lower DSI indices than Spunta. Saba *et al.* (2001) proposed that narrow-sense heritability for

GMP and DTI were higher compared to other drought tolerance indices, suggesting that these indices could be useful for breeding selection of drought tolerant genotypes. Dry matter content in tuber of both cultivars was not affected by drought treatment, in agreement with previous results (Cabello *et al.*, 2012; Deblonde and Ledent, 2000). Under the drought conditions applied in this study, Spunta was the cultivar showing the higher shoot dry biomass and while Newen INTA exhibited the higher root dry biomass. Increased foliage development was described in drought-sensitive potato cultivars (Aliche *et al.*, 2018). On the other hand, a large root system is likely a mechanism of adaptation to drought in potato (Boguszewska-Mańkowska *et al.*, 2020). Hence, R/S ratio is a suitable indicator of drought tolerance since a higher R/S ratio improves soil water uptake and reduces water losses for transpiration (Zhang *et al.*, 2018). When comparing Spunta and Newen INTA, R/S was not modified significantly under drought stress. Root biomass was associated with potato yield under drought (Zarzynska *et al.*, 2017), and the correlation between marketable yield and dry root biomass observed in Newen INTA and Spunta agrees with this idea.

Drought treatment increased proline content in both cultivars. However, the proline content in Spunta was higher than in Newen INTA across the whole cycle. Claussen (2005) described that the content of foliar proline might be considered as an indicator of drought stress. However, the role of proline content in potatoes under drought conditions was controversial (Ashraf and Foolad, 2007; Schafleitner *et al.*, 2007). The proline accumulation might contribute to drought tolerance or indicate stress intensity. According to Tagliotti *et al.* (2020) under moderate drought stress, proline accumulation

was not associated with drought tolerance. The observed negative correlation between proline content and the number of marketable tubers suggests that proline content could be an indicator of stress, rather than a stress protector (Schafleitner *et al.*, 2007; Tagliotti *et al.*, 2020). Even significantly higher proline level was observed in the leaves of different species, proline deposition in the root-tip of maize, barley and watermelon (among others) was described under low water potential. However, this increase in proline root content was not due to the proline biosynthesis in the roots but rather its increased transport from leaves (Verslues and Sharp, 1999; Raymond and Smirnoff, 2002; Shelden *et al.*, 2016; Wang *et al.*, 2022). Probably, the negative correlation observed in this study between proline content and the number of marketable tubers could be due to most of the proline synthesized in leaves was already mobilized to root at the time of sampling.

Evaluation of the effect of drought on physiological, growth and yield parameters suggested that Newen INTA is a drought tolerant cultivar respect to Spunta, for which can be considered a good genotype for improving crop production in semi-arid regions.

## CONCLUSIONS

Two elite potato cultivars in Argentine showed a differential drought tolerance. Newen INTA presented an improved drought tolerance respect to Spunta. The drought tolerance indices used (GMP, DTI, and DSI) were suitable to address drought tolerance. The role of proline content was as an indicator of non-specific stress, rather than an indicator of drought tolerance. The present results propose Newen INTA as a promising potato cultivar to improve potato production and to save irrigation resources.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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# PERFORMANCE OF DIFFERENT MULCHES ON PHYSICAL GROWTH AND YIELD OF POTATO (*SOLANUM TUBEROSUM* L.) IN WESTERN RWANDA

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**ABSTRACT:** The objective of this study was to investigate the effect of various types of mulches on physical growth and yield of potatoes (*Solanum tuberosum* L.). The experiments were conducted in farmer's fields during the agricultural seasons 2022 B (Season B is from March to June) and 2023 A (Season A is from September to February). Experiment was laid out in a Randomized Complete Block Design (RCBD) in two sites with four replications and five treatments: T1 (Control), T2 (Dead mulch), T3 (Living mulch of Mucuna), T4 (Living mulch of Desmodium), and T5 (Living mulch of Lablab). Data were collected on the potato physical growth with an interval of 15 days after planting (DAP) while yield data were collected immediately after harvesting. The Analysis of Variance (ANOVA) was done in Microsoft Excel at  $P < 0.05$ . Results revealed a significant difference in physical growth of plants in all treatments with steady increase up to 90 DAP and decrease at 105 DAP in both Districts. Yield average was 37.67 t/ha and 30.83 t/ha in Rubavu and Nyabihu Districts respectively. It was the highest for dead mulch at 57.6 t/ha in Rubavu and 43.0 t/ha in Nyabihu District. It was followed by Lablab spp at 42.3 t/ha for Rubavu and 37.3 t/ha for Nyabihu District. Yield increment was 42.0 % and 3.8 % in Nyabihu and Rubavu Districts respectively. Dead mulch and Lablab contributed more to the yield increase and can be recommended as farming practice to increase potato yield in both Districts.

**KEYWORDS:** Mulches, Physical growth, Potato, yield increment

## INTRODUCTION

Worldwide, potato (*Solanum tuberosum* L.) is one of the most consumable vegetable crops. At present, potato is grown in more than 160 countries in the world and is considered as one of the four major agronomic crops (Donnelly and Kubow, 2011). Potato originated from the highlands of South America and discovered by Spanish explorers. Thereafter, it spread globally over the last years. Potato was introduced in Europe in the late 16th century (FAO, 2020) and around the turn of the 20th century, the potato arrived in Africa (FAO, 2020). It was introduced

in Rwanda by Germany missionaries and Rwanda is the sixth largest producer of potato in Africa (FAO, 2019). Currently potato is cultivated and consumed all over the world.

Potato is a non-grain crop and has gained much importance around the globe. More than a billion people worldwide eat potatoes, and produced globally over 300 million metric tons (CIP, 2023). In Rwanda, potato covers 3.9 % of total cultivated land per each growing season and shares 10 % of total main crop production (MINAGRI, 2019). Most of the potato producers consists of small family farms that intercrop potatoes

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with beans and maize, and the productivity is still low around 10 t/ha compared to other countries such as Netherlands and German with productivity of 35 t/ha (MINAGRI, 2019; RAB, 2021). National Institute of Statistics of Rwanda (NISR,) indicated that the per capita consumption of potato is 125 kg per year (MINAGRI, 2019). In Rwanda, potato is the third major crop after cassava and sweet potato, in terms of volume of production, and it became an important cash crop in the Northern volcanic areas, highlands of Buberuka, and the Congo/Nile Divide (MINAGRI, 2019). This Northern volcanic region accounts for more than 80% of the national potato production, while the rest is produced on marginal plantations throughout the country (MINAGRI, 2019). In this highland region, potato is the staple food with more than 60% of the production being used directly for home consumption (MINAGRI, 2019). It has high rate of production and it is also considered as a cash crop.

Potato production in Rwanda faces many challenges which hinder its effective productivity. The main challenges causing this low potato productivity include small and fragmented potato production land, poor linkage of potato producers and markets, limited access to credit for agriculture inputs, shortage of appropriate post-harvest handling and processing technologies, inadequate supply of high-quality seeds to farmers, low rate of fertilizer use, irrigation, pests and diseases problems (Rukundo *et al.*, 2019). Availability of improved and high yielding potato varieties adapted to current biotic and abiotic stresses is worsened by the current climate change (RAB, 2021). Productivity of potato is constrained by many factors which can be dealt with to optimize potato production.

Conventional methods of potato production are facing different challenges in

potato production. It is common to increase production through fertilization and also irrigation system. However, there are other factors which are not given much attention, but which contribution cannot be ignored. It was reported that fluctuation of soil moisture negatively affects seed germination and emergence which in return affects production (Ahmed *et al.*, 2017; Dvořák *et al.*, 2012). Potato production requires moderate temperature, while extremely hot or cold climate affect potato production (Lia *et al.*, 2017). The optimum yield is obtained at temperature range of 15-20°C and the growth reduces at the temperature below 15°C and above 20°C (Lia *et al.*, 2017). Potato production is also affected by growth of weeds which compete with the main crop and reduce production (Ferdous *et al.*, 2017). More importantly, given the relief of potato farms in Rwanda, erosion is commonplace among threatening factors in potato production. Therefore, it is essential to implement productivity-boosting strategies in order to enhance potato production.

Mulching is among improved cultivation practices to increase the yield and productivity of potatoes to its maximum. According to Bharati (2020), mulching has a significant influence on the growth and yield of potatoes. Other scientists reported that mulching promotes soil water infiltration and crop water availability which helps to improve soil biodiversity and environmental benefits (Christianson *et al.*, 2017; Waheed *et al.*, 2023). Mulches function as cover crops to reduce tillage operations that have some ecological advantages over conventional land preparation tasks causing minimum alterations in the soil environment (Christianson *et al.*, 2017). This contributes to higher water use efficiency by plants, higher yields, and greater economic benefits. Mulches were also reported to improve soil structure, control temperature fluctuation,

control weeds and preserve nutrients which contribute to roots growth as well as plant's overall health (Ahmed *et al.*, 2017; Dvořák *et al.*, 2012). Therefore, the aim of this study was to investigate the effect of different types of mulches on physical growth and yield of potato grown in western Rwanda.

## MATERIALS AND METHODS

### Experimental site

The present experiment was conducted in farmer's fields at two different sites of Rurengeri Cell, Mukamira sector in Nyabihu District, and in Muhira cell, Rugerero sector in Rubavu District from September 2022 to January 2023. Nyabihu District is located on a volcanic mountain with relief characterized by 90 % rugged mountains with slopes of more than 55 %, creating a high risk of erosion. Soils comprise sandy and clay, laterite, and volcanic. Precipitation is almost uniform over every month and close to 1400 mm per year. It has a climate with an average temperature of 15°C (Nyabihu District, 2019). Rubavu District is located in a volcanic mountain with a soil characterized by volcanic lava soil. The average temperature range is 15-20°C with the annual rainfall varying from 1200 mm to 1500 mm (Rubavu District, 2019).

### Experimental design and field layout

In both Districts (Nyabihu and Rubavu) the experiment was laid out in Randomized Complete Block Design (RCBD) with four replications and five treatments during the period from September 2022 to January 2023. The field experiment at each site was composed of 20 experimental plots of dimensions 4 m × 3 m with spacing 1m within the blocks and 2 m between the blocks. Five treatments were assigned to each block to study the effect of various types of mulches and cover crops on the physical growth and yield of potato.

The treatment details:

- T1: Control,
- T2: Dead Mulch (straw of beans) (5 t/ha),
- T3: Living Mulch of Mucuna (seeds of 50 kg/ha),
- T4: Living Mulch of Desmodium (seeds of 50 kg/ha), and
- T5: Living Mulch of Lablab (seeds of 50 kg/ha).

The planting density of potato was 80 × 30 cm between and within rows respectively, which gave a total of 50 planting holes per elementary plot, and 41,667 plants/ha. Intercropped with cover crops of Lablab spp, Desmodium spp, Mucuna spp separately in each experimental units and dead mulch of straw of beans was obtained from farmers harvest of the previous agricultural season. The potato seeds of the Kinigi variety and cover crops of Lablab spp, Desmodium spp, and Mucuna spp were obtained from Rwanda Agriculture Board (RAB).

### Data collection

Data was collected from randomly selected 5 plants from each plot excluding border plants, and the obtained data was used to measure various growth and yield parameters. The data on vegetative growth, plant biomass (number), plant height (cm), and plant diameter (cm) were collected at the period interval of 15 Days After Planting (DAP) starting from 45 to 105 DAP, while yield data was taken after harvesting at the end of the season both at Nyabihu and Rubavu Districts. Technical data collection sheets, rulers, weighing scales, and pair calipers were availed for that purpose. All data on the physical growth and yield were collected at the same sampled plant from all experimental units.

## Statistical data analysis

Collected data were subjected to analysis of variance (ANOVA) appropriate to the RCBD in Microsoft Excel at  $P < 0.05$  levels of probability test and the treatment means were separated into homogeneous groups using the Least Significant Difference (LSD).

## RESULTS AND DISCUSSION

### Plant leaf development (number)

The results of the ANOVA showed that there was a significant difference in number of leaves in both sites and in all treatments from 45 DAP to 105 DAP at  $P < 0.05$  as depicted in Tables 1 and 2. In both sites and all treatments, the number of leaves increased progressively up to 90 days and it reduced

afterwards. The reduction of leaves after 90 DAP might have been caused by falling of leaves due to maturity with no replacement of new leaves because of approaching maturity stage. Number of leaves was higher for dead mulch and lower for Desmodium and control. The increase in number of leaves for dead mulch might have been caused by their function as fertilizer. In general, number of leaves in Rubavu was higher than in Nyabihu District. Living mulches were reported to provide organic matter to crops and on the other hand can compete with targeted crops (Christianson *et al.*, 2017). Effect of different mulches on increase of number of leaves was also reported by Bharati (2020) where mulches influenced leaves development with steady increase to the maximum up to 75

**Table 1: Plant leaf development (number) from 45 to 105 DAP in Nyabihu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	22.10 <sup>cd</sup>	43.95 <sup>d</sup>	112.80 <sup>cd</sup>	143.60 <sup>bcd</sup>	66.25 <sup>d</sup>
Dead Mulch	32.60 <sup>a</sup>	60.45 <sup>a</sup>	185.51 <sup>a</sup>	219.67 <sup>a</sup>	108.12 <sup>a</sup>
Desmodium spp	22.07 <sup>d</sup>	51.35 <sup>bcd</sup>	109.40 <sup>d</sup>	138.80 <sup>cd</sup>	68.50 <sup>cd</sup>
Lablab spp	27.50 <sup>abcd</sup>	53.30 <sup>abc</sup>	146.00 <sup>abcd</sup>	171.75 <sup>abcd</sup>	86.15 <sup>abcd</sup>
Mucuna spp	23.30 <sup>bcd</sup>	48.20 <sup>cd</sup>	113.45 <sup>bcd</sup>	134.41 <sup>d</sup>	70.95 <sup>bcd</sup>
General Mean	25.51	51.45	133.43	161.64	79.99
CV	14.11	3	13.91	13.32	14.01
LSD (5%)	8.69	8.92	53.24	60.05	29.47

Means followed by the same letter(s) in a column are not significantly different by LSD at 5 % level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

**Table 2: Plant leaf development (number) from 45 to 105 DAP in Rubavu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	21.95 <sup>d</sup>	45.30 <sup>d</sup>	111.50 <sup>cd</sup>	147.61 <sup>bcd</sup>	70.25 <sup>d</sup>
Dead Mulch	39.28 <sup>a</sup>	61.85 <sup>a</sup>	184.26 <sup>a</sup>	223.67 <sup>a</sup>	112.13 <sup>a</sup>
Desmodium spp	22.21 <sup>cd</sup>	49.85 <sup>bcd</sup>	124.40 <sup>bcd</sup>	141.30 <sup>cd</sup>	72.01 <sup>bcd</sup>
Lablab spp	29.35 <sup>abcd</sup>	55.10 <sup>abc</sup>	161.00 <sup>abc</sup>	174.25 <sup>abcd</sup>	89.65 <sup>abcd</sup>
Mucuna spp	23.38 <sup>bcd</sup>	46.70 <sup>cd</sup>	76.15 <sup>d</sup>	134.40 <sup>d</sup>	70.95 <sup>cd</sup>
General Mean	27.23	51.75	131.45	164.24	82.99
CV	0.024	0.03	0.14	0.13	0.14
LSD (5%)	10.5	9.77	65.42	61.16	30.23

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

DAP. Contribution on quality of potatoes due to dead mulch was also reported by Lia *et al.* (2017) where it was also site dependent. Potatoes grow better at the temperature range of 15-20°C and the growth reduces at the temperature below 15°C and above 20°C Lia *et al.* (2017) which might have caused better development of leaves in Rubavu than Nyabihu. Living and dead mulch contribute differently on the increase in number of leaves.

### Plant Height (cm)

The results of the ANOVA showed that there was no significant difference in plant height for 45 DAP and 60 DAP in Nyabihu District and at 60 DAP in Rubavu District. However, the significant difference was

noticed from 75 DAP to 105 DAP in Nyabihu District and in Rubavu District it was from 45 DAP to 105 DAP except for 60 DAP at  $P < 0.05$  as depicted in Tables 3 and 4. There was a steady increase in plant height of potatoes from both sites and all treatments from 45 DAP to 90 DAP followed by reduction in plant height at 105 DAP. Reduction in plant height at 105 DAP may be related to reduction of sap supply due to maturity which causes shrinkage of growth tissues. Dead mulch and Lablab spp contributed more in plant height in Nyabihu District, while in Rubavu District the highest contribution was for dead mulch. The control had the lowest contribution in both sites. Plant height was higher in Rubavu District than Nyabihu District. Increase in height of potatoes due to

**Table 3: Plant height (cm) from 45 to 105 DAP in Nyabihu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	39.84 <sup>a</sup>	50.31 <sup>a</sup>	70.15 <sup>b</sup>	81.23 <sup>b</sup>	77.93 <sup>b</sup>
Dead Mulch	48.47 <sup>a</sup>	63.63 <sup>a</sup>	85.75 <sup>a</sup>	101.33 <sup>a</sup>	99.09 <sup>a</sup>
Desmodium spp	41.47 <sup>a</sup>	55.52 <sup>a</sup>	78.35 <sup>ab</sup>	91.18 <sup>ab</sup>	87.88 <sup>ab</sup>
Lablab spp	43.47 <sup>a</sup>	62.48 <sup>a</sup>	81.57 <sup>a</sup>	94.25 <sup>a</sup>	91.85 <sup>a</sup>
Mucuna spp	41.63 <sup>a</sup>	61.13 <sup>a</sup>	78.83 <sup>ab</sup>	91.95 <sup>a</sup>	88.65 <sup>a</sup>
General Mean	42.95	58.6	78.92	91.99	89.08
CV	9.8	12.04	6.59	6.72	7.01
LSD (5%)	10.14	13.50	11.22	14.72	15.07

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5 % level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

**Table 4: Plant height (cm) from 45 to 105 DAP in Rubavu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	41.90 <sup>bcd</sup>	52.61 <sup>b</sup>	72.35 <sup>c</sup>	84.23 <sup>b</sup>	80.83 <sup>b</sup>
Dead Mulch	50.37 <sup>a</sup>	65.93	87.95 <sup>a</sup>	104.33 <sup>a</sup>	101.99 <sup>a</sup>
Desmodium spp	41.80 <sup>cd</sup>	56.32 <sup>ab</sup>	85.95 <sup>ab</sup>	92.38 <sup>ab</sup>	90.28 <sup>ab</sup>
Lablab spp	42.57 <sup>abcd</sup>	63.20 <sup>b</sup>	89.11 <sup>a</sup>	95.45 <sup>ab</sup>	94.25 <sup>ab</sup>
Mucuna spp	38.41 <sup>d</sup>	58.03 <sup>ab</sup>	73.93 <sup>bc</sup>	91.95 <sup>ab</sup>	88.65 <sup>ab</sup>
General Mean	43.01	59.22	81.86	93.67	91.2
CV	0.098	0.12	0.06	0.07	0.07
LSD (5%)	8.39	13.71	13.8	14.76	15.19

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

the influence of mulches was also reported by (Bharati, 2020). Similarly, living mulches were reported to increase plant biomass where leguminous mulches provide more nitrogen to the soil there by contributing to the growth of targeted crop (Christianson *et al.*, 2017). Living and dead mulch have influence on the plant height.

### Plant stem diameter (Cm)

The results from ANOVA revealed that there was no significant difference in plant diameter from Nyabihu District at 45 and 75 DAP while the significant difference was noticed at 60, 90 and 105 DAP and in all treatments of Nyabihu District, while in Rubavu District the difference was significant from 45 to 105 DAP at  $P < 0.05$  as presented

in Tables 5 and 6. There was a steady increase in diameter of plant in all treatments up to 90 DAP. Thereafter, the diameter reduced. The reduction in diameter might have been caused by shortage of sap supply to the plant followed by its shrinkage. Dead mulch contributed more to the increase in plant diameter while the least contribution was for the control. In general plant diameter in Rubavu District was higher than in Nyabihu District. Increase in stem diameter due to mulches was also report by (Bharati, 2020). Living mulch were also reported in reducing leaching of nutrients which in turn can contribute to the growth of the main plant (Christianson *et al.*, 2017). Growth diameter of potato stem was influenced differently by both living and dead mulches.

**Table 5: Plant stem diameter (cm) from 45 to 105 DAP in Nyabihu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	1.26 <sup>a</sup>	1.33 <sup>b</sup>	1.36 <sup>a</sup>	1.33 <sup>b</sup>	0.84 <sup>c</sup>
Dead Mulch	1.39 <sup>a</sup>	1.47 <sup>a</sup>	1.5 <sup>a</sup>	1.53 <sup>a</sup>	0.99 <sup>a</sup>
Desmodium spp	1.33 <sup>a</sup>	1.38 <sup>ab</sup>	1.42 <sup>a</sup>	1.38 <sup>ab</sup>	0.86 <sup>bc</sup>
Lablab spp	1.37 <sup>a</sup>	1.43 <sup>a</sup>	1.5 <sup>a</sup>	1.49 <sup>a</sup>	0.92 <sup>abc</sup>
Mucuna spp	1.33 <sup>a</sup>	1.39 <sup>ab</sup>	1.44 <sup>a</sup>	1.41 <sup>ab</sup>	0.91 <sup>abc</sup>
General Mean	1.33	1.4	1.45	1.42	0.9
CV	4.85	2.46	4.63	3.86	4.8
LSD (5%)	0.14	0.11	0.15	0.13	0.11

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5 % level of significance, CV=Coefficient of variation, LSD = Least Significant Differences, DAP = Days After Planting

**Table 6: Plant stem diameter (cm) from 45 to 105 DAP in Rubavu District**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP
Control	1.29 <sup>bc</sup>	1.39 <sup>bc</sup>	1.46 <sup>a</sup>	1.64 <sup>bc</sup>	0.84 <sup>e</sup>
Dead Mulch	1.58 <sup>a</sup>	1.53 <sup>a</sup>	1.60 <sup>a</sup>	1.81 <sup>ab</sup>	1.19 <sup>a</sup>
Desmodium spp	1.36 <sup>ab</sup>	1.35 <sup>c</sup>	1.55 <sup>a</sup>	1.91 <sup>ab</sup>	0.89 <sup>de</sup>
Lablab spp	1.43 <sup>ab</sup>	1.40 <sup>abc</sup>	1.57 <sup>a</sup>	2.02 <sup>a</sup>	0.95 <sup>bcde</sup>
Mucuna spp	1.08 <sup>c</sup>	1.45 <sup>ab</sup>	1.13 <sup>b</sup>	1.41 <sup>c</sup>	0.91 <sup>cde</sup>
General Mean	1.35	1.42	1.45	1.76	0.96
CV	4.8	2.4	4.6	3.1	4.5
LSD (5%)	0.28	0.13	0.28	0.35	0.20

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

## Influence of mulches potato yield

The results of the ANOVA showed a comparative response of different mulches and cover crops on the yield per plant and yield per hectare in Rubavu and Nyabihu Districts as depicted in Tables 7 and 8. There was a significant difference in plant yield for all treatments in all sites at  $P < 0.05$ . The highest plant yield in both sites was for dead mulch with more than 1 kg per plant followed by Lablab spp, control was the least in Nyabihu District while Mucuna was the least in Rubavu District. In general plant

**Table 7: Comparison of the yield per plant (kg) in Nyabihu and Rubavu Districts**

Treatment	Yield /Plant (kg) Nyabihu District	Yield /Plant (kg) Rubavu District
Control	0.52 <sup>d</sup>	0.87 <sup>bcd</sup>
Dead Mulch	1.03 <sup>a</sup>	1.38 <sup>a</sup>
Desmodium spp	0.59 <sup>cd</sup>	0.72 <sup>cd</sup>
Lablab spp	0.89 <sup>abc</sup>	1.02 <sup>abc</sup>
Mucuna spp	0.65 <sup>bcd</sup>	0.53 <sup>d</sup>
General Mean	0.74	0.90
CV	10.72	8.8
LSD (5%)	0.33	0.47

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5 % level of significance, CV=Coefficient of variation, LSD=Least Significant Differences

**Table 8: Comparison of the yield (t/ha) from various mulches and cover crops in Rubavu and Nyabihu District**

Treatments	Yield (t/ha) Rubavu District	Yield (t/ha) Nyabihu District
Dead Mulch	57.63 <sup>a</sup>	43.05 <sup>a</sup>
Lablab spp	42.34 <sup>b</sup>	37.34 <sup>b</sup>
Control	36.30 <sup>c</sup>	21.72 <sup>d</sup>
Desmodium spp	29.79 <sup>d</sup>	24.79 <sup>c</sup>
Mucuna spp	22.29 <sup>e</sup>	27.29 <sup>c</sup>
General Mean	37.67	30.84
CV	4.77	5.08
LSD	2.53	2.71

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences

yield was higher in Rubavu than in Nyabihu District.

Yield per hectare in all treatments was evaluated. There was a significant difference in yield of all treatments at  $P < 0.05$  in both Districts as depicted by Tables 7 and 8. Dead mulch and Lablab spp showed the highest yield in both Districts and the yield was higher than the control. In Rubavu District, control was higher than Desmodium spp and Mucuna spp where Mucuna spp showed the lowest yield in this District. Moreover, in Nyabihu District, the lowest yield was for the control and the control in Nyabihu District had the lowest yield in both Districts followed by Mucuna spp in Rubavu District. Overall, the highest yield was in Rubavu with yield of 37.67 t/ha and 30.84 t/ha for Nyabihu District. These results are very high comparing to the existing national yield target of potato which is 14 t/ha in comparison with the current yield of 8.6 t/ha obtained from existing farming techniques as stated by Rukundo (2019) and MINAGRI (2019). Therefore, the technique of mulching can be adopted to increase potato productivity. Special consideration should be given to dead mulch and Lablab which were found to increase productivity to the highest level.

## Potato yield increment

Yield increment was calculated based on the control as a reference and reported in percentage as shown in Figure 1 below. In Nyabihu District there was yield increment of 98.20 % for dead mulch, 71.90 % for Lablab, 25.70 % for Mucuna and 14.10 % for Desmodium with the average yield increment of 42.00 %. On the other hand, yield increment in Rubavu District was 58.80 % for Dead mulch, 16.60 % for Lablab, negative 38.60 % for Mucuna, and negative 17.90 % for Desodium where general increment was 3.80 %. Yield increment in Rubavu was lower than the

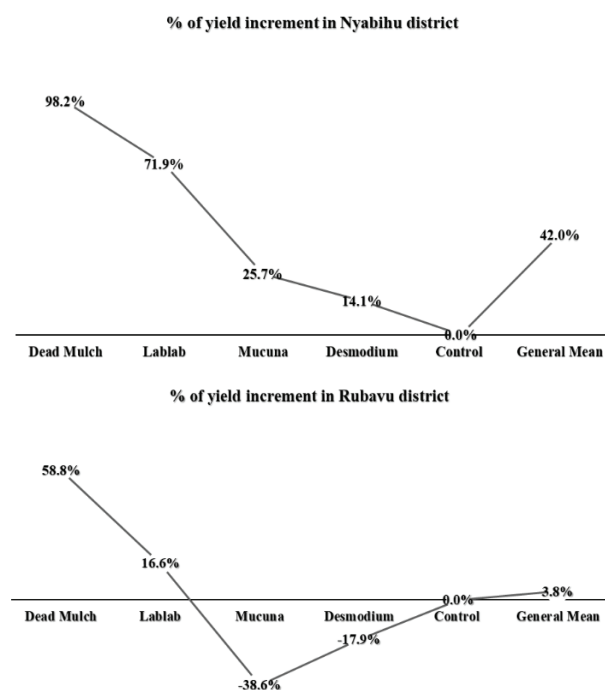


Fig.1. Effect of mulch and cover crops on yield increment (t/ha) of Irish potatoes in Nyabihu and Rubavu Districts

one of Nyabihu District where general yield increment was 42.00 % in Nyabihu District compared to 3.80 % of Rubavu District. Low yield increment in Rubavu was due to that the control in that District performed better than the control of Nyabihu even more than some other treatments. In Rubavu District Mucuna and Desmodium affected negatively the yield of potato where their yield was less than the control. Yield increment using different mulches was also reported by (Bharati, 2020) where they influenced yield differently. Negative increment in potato yield for Desmodium and Mucuna in Rubavu District might have been caused by their competition with the main plant. Negative impact of living mulches on crop yield was also reported by Lia *et al* (2018). Variation in yield may also be influenced by other factor like soil characteristics, rainfall and temperature. Therefore, dead mulch and Lablab can be recommended as suitable mulches in both Districts.

## CONCLUSION

Potato is very sensitive to various mulch and cover crops in terms of physical growth and yield. The results revealed that from 45 to 105 DAP, there was a significant difference and a steady increase among treatment means in Nyabihu and Rubavu Districts in physical growth. Among all treatments, dead mulch and Lablab spp, showed the highest potentiality and suitability in mulching potatoes by comparing to others like Mucuna, Desmodium spp and untreated plots in increasing potato yield. Mucuna and Desmodium affected negatively the yield of potato in Rubavu District. Living mulch might have competed with potatoes on the nutrients. Soil characteristics and climatic conditions might have influenced growth in addition to mulches. Therefore, dead mulch and Lablab spp can be recommended for mulching in both Districts. Furthermore, it is recommended to investigate the effect of different types of mulches on soil properties to reveal the suitable mulch for each type of soil and climate. Cost-benefit analysis should also be conducted to investigate whether implementing these techniques will provide enough income for farmers to change their livelihood.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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# ANALYSIS OF CAROTENOIDS METABOLISM GENES LANDSCAPE IN POTATO AND CLONING OF ZEAXANTHIN EPOXIDASE GENE

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**ABSTRACT:** Genome-wide identification of all the genes involved in carotenoids biosynthesis were carried out *in silico* using potato genome sequence. Of the 12 genes analysed [geranylgeranyl pyrophosphate synthase (GGPS), phytoene synthase (PSY), f-carotene desaturase (ZDS), carotenoid isomerase (CRTISO), lycopene  $\epsilon$ -cyclase (LCY- $\epsilon$ ), lycopene  $\beta$ -cyclase (LCY- $\beta$ ),  $\beta$ -carotene hydroxylase (CHY- $\beta$ ), violaxanthin de-epoxidase (VDE), zeaxanthin epoxidase (ZEP), neoxanthin synthase (NXS), carotenoid cleavage dioxygenase (CCD), 9-cis-epoxycarotenoids dioxygenase (NCED)]; three genes namely ZDS, CRTISO and LCY- $\epsilon$  was found as single gene, on chromosome 1, 10 and 12, respectively. GGPS (chromosomes 4 & 11), LCY- $\beta$  (chromosomes 4 & 10), CHY- $\beta$  (chromosomes 3 & 6) had two genes each. VDE (chromosome 4), NXS (chromosomes 2, 3 & 6) and NCED (chromosome 5, 7 & 8) showed presence of 3, 4 and 5 genes, respectively. CCD (chromosome 1, 7 & 8) and PSY (chromosomes 2, 3, 4, 8 & 12) had six genes each in potato genome. These carotenoid biosynthesis genes differed in terms of number of introns, length of open reading frame, molecular weight and isoelectric points of deduced proteins, secondary structure composition etc. This information generated about genes and proteins of carotenoids biosynthesis pathway in potato may be very crucial and of vital utility for genome editing mediated interventions for enhancing total/targeted carotenoids content in potato. Further, ZEP was cloned full length. The complete ORF of cloned ZEP was 2010 nucleotides long and encoding a protein having 669 amino acids (NCBI acc no. MK852682). It exhibited variations at 9 amino acid positions when compared with the one available in potato genome sequence database. These variations at 9 amino acids might provide some uniqueness to the cloned gene which might be involved in regulation of carotenoid biosynthesis pathway especially beta-carotene, violaxanthin, zeaxanthin carotenoids etc.

**KEYWORDS:** Genome-wide, genome editing, gene structure, metabolic engineering

## INTRODUCTION

Humans and animals do not synthesize carotenoids and thus mainly depends on plant-based diets as source of carotenoids. Carotenoids are necessary to maintain normal health and behavior of animals. Carotenoids acts as precursors of vitamin A and play a range of other important functions in human health. Consumption of carotenoids has been associated with beneficial effects in a number of systemic diseases, eye disorders and cognitive function (Tapiero *et al.*, 2004; Lima *et al.*, 2016; Buscemi *et al.*, 2018; Eggersdorfer and

Wyss, 2018). Of more than several hundred carotenoids found in nature only about 50 carotenoids are found in majority of human diet, and around 20 of them are present in human tissue and blood (Fiedor and Burda, 2014). The carotenoids primarily exert antioxidant effects, but individual carotenoids may also act through other mechanisms.

In plants, carotenoids and their oxidative and enzymatic cleavage products are crucial for various biological processes in plants, such as assembly of photosystems and light harvesting antenna complexes

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for photosynthesis and photoprotection, and regulation of growth and development (Cazzonelli and Pogson, 2010; Ruiz-Sola and Rodriguez-Concepcion, 2012; Havaux, 2014). Carotenoids are also reported to act as signalling molecules and are thus involved in plant- environment interactions (Walter and Strack, 2011; Cazzonelli, 2011). Due to the pivotal role of carotenoids in nature, the understanding of how plant cells regulate the accumulation and flux of various carotenoids and their metabolites is advancing rapidly. Yet, further insights are essentially required for making plants synthesise desired carotenoids in higher quantities to make them nutritionally more enriched. Carotenoid are C40 tetraterpenoid lipophilic metabolites and are derived from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). The IPP and DMAPP used for carotenoid biosynthesis in plants are derived from the MEP pathway (Rodriguez-Concepcion and Boronat, 2002). Various enzymes involved in the carotenoids biosynthesis pathway are depicted in Fig. 1.

In potato tubers total carotenoid content ranges between 0.05 to 0.6 mg per 100 g fresh weight (FW) (Brown, 2005; Xu *et al.*, 2003). However, few studies have also reported carotenoid content in potato beyond this range (Bonierbale *et al.*, 2009; Tatarowska *et al.*, 2019; Bahadori *et al.*, 2023). The major carotenoids in potato include; zeaxanthin, antheraxanthin, violaxanthin and lutein, whereas neoxanthin,  $\beta$ -carotene and  $\beta$ -cryptoxanthin are found in trace amounts (Burgos *et al.*, 2009, Bonierbale *et al.*, 2009). However, their relative proportions have been shown to be varying. Tuber carotenoids content is affected by genotype and growing environment (Hamouz *et al.*, 2016). Also, it has been found that total carotenoids content is higher in immature tubers and decreases with tuber maturity (Kotikova *et al.*, 2007).

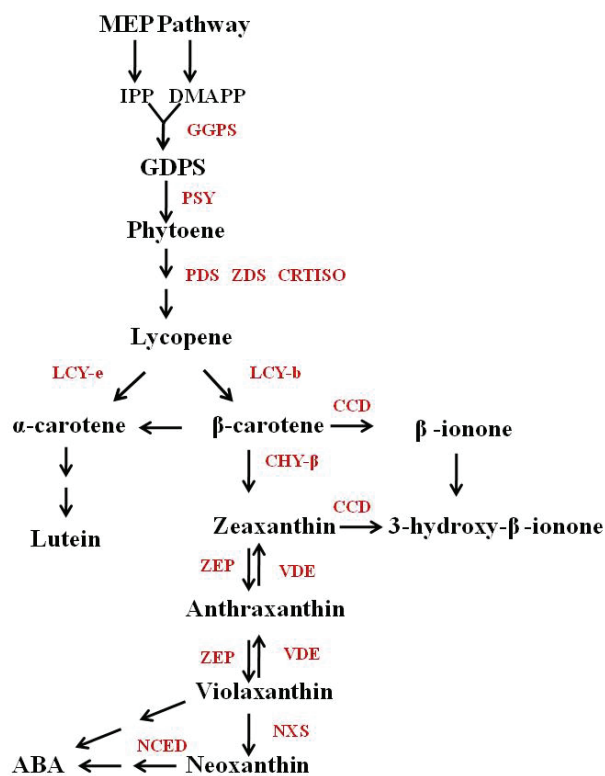


Fig. 1: Enzymes of carotenoids biosynthesis pathway in plants. GGPS: geranylgeranyl pyrophosphate synthase. PSY: phytoene synthase; PDS: phytoene desaturase; ZDS:  $\beta$ -carotene desaturase; CRTISO: carotenoid isomerase; LCY- $\epsilon$ : lycopene  $\epsilon$ -cyclase; LCY  $\beta$ , lycopene  $\beta$ -cyclase; CHY- $\beta$ ,  $\beta$ -carotene hydroxylase; VDE, violaxanthin de-epoxidase; ZEP, zeaxanthin epoxidase; NXS, neoxanthin synthase; CCD, carotenoid cleavage dioxygenase; NCED, 9-cis-epoxycarotenoids dioxygenase.

Numerous groups have attempted to increase potato carotenoids using transgenic strategies. The strategy commonly used in plants is to increase the biosynthetic capacity by altering the carotenogenic enzyme activities (Romer *et al.*, 2002, Ducreux *et al.*, 2005; Morris *et al.*, 2006; Diretto *et al.*, 2007) but due to stringent regulations for genetically modified organisms these transgenic plant lines could not reach the farms and thus were not cultivated. However, now with the advent of advanced genome editing tools and the fact that in most of the countries the plants developed using genome editing approaches (Site-Directed Nuclease 1 (SDN1)

and Site-Directed Nuclease 2 categories) have been kept out of the ambit of bio-safety regulations and thus most probably are speculated reaching fields and the consumers in near future. Having prior knowledge about the molecular machinery (genes and proteins) associated with the trait aimed to be improved using genome editing approach, is pre-requisite. Keeping this in view, the present study was undertaken on genome-wide analysis of carotenoid metabolism in potato (*Solanum tuberosum* L.). Further, an important gene of the carotenoid pathway was cloned from potato and characterized.

## MATERIALS AND METHODS

### Planting Material

Well sprouted tubers of Kufri Jyoti cultivar of potato were planted in pots and were kept in plant growth chambers having 22 / 18 °C day/ night temperature with 16 h photoperiod. Leaves at 4<sup>th</sup> position from the top were used for extraction of RNA.

### Cloning of cDNA of zeaxanthin epoxidase

RNA was isolated from leaf tissue using PureLink®RNA Mini Kit (M/s

Lifetechnologies, USA) and digested with DNase I (RNase free) (M/s Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesized from 2 µg of DNase-treated total RNA as a template in 20 µl reaction volume by using cDNA synthesis kit (Invitrogen, USA). Primers (O6629-F-F1 and O6629-F-R1) for ZEP were designed from the conserved regions of corresponding gene reported from different plant sources and the partial gene sequence was amplified by PCR as detailed in Table 1. Primers were designed by using Primer3 (<http://primer3.ut.ee>) and got synthesized from M/s Integrated DNA Technologies (IDT) considering important factors like % GC content, melting temperature for the primer set, and formation of internal hairpin loops and dimerization of oligos. List of primers used are listed in Table 1. The amplicon was cloned in pGEM-T Easy Vector (Promega, USA), plasmids were isolated using GeneJET™ Plasmid Miniprep Kit (M/s Fermentas Inc., USA), and sequencing was performed using Big Dye terminator cycle sequencing mix (Version 3.1; M/s Applied Biosystems, USA) using an automated DNA sequencer (ABI 3130 xl Genetic Analyzer, M/s Applied Biosystems, USA). Protocols

Table 1: Primer used for cloning and expression analysis

S. No.	Name of primer	Sequence of primers (5'-3')	PCR condition
Primer used for cloning of full length ORF			
1	06629-F-F5	ATGTATTCAACTGTGTTTTACACTTC	Initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C, 30 s; 55–60 °C, 40 s; 72 °C, 50 s. Final extension at 72 °C for 7 min
2	06629-F-R5	GTGAAGCAGTGGGGGCAGCGTAA	
3	O6629-F-F1	ATGTATTCAACTGTGTTTTACACTTC	Initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C, 30 s; 55–60 °C, 40 s; 72 °C, 50 s. Final extension at 72 °C for 7 min
4	06629-F-R1	CATCACTGGTCAAAGGATTA	
5	06629-F-F2	CATCACTGGTCAAAGGATTA	
6	06629-F-R2	CTTGTTTCTTCAGATGTGG	
7	06629-F-F3	CTTGTTATACTGGAATTGCA	
8	06629-F-R3	GAATGGCTGCAATCATGGCA	
9	06629-F-F4	GAATGGCTGCAATCATGGCA	
10	06629-F-R4	GTGAAGCAGTGGGGGCAGCGTAA	

were followed essentially as described by the respective manufacturer. For sequencing, full length cloned CDS of ZEP, the primers were designed (06629-F-F2, 06629-F-R2, 06629-F-F3, 06629-F-R3, 06629-F-F4, 06629-F-R4) in order to get overlapping fragments amplified which were sub-cloned and sequenced. Sequences of the sub-cloned fragment obtained were overlapped to derive the complete sequence of cloned ORF.

### **Genome-wide analysis of carotenoids metabolism pathway genes**

Carotenoid pathway genes were searched in various published literature and were then looked into potato genome sequence database (<http://spuddb.uga.edu/>) using PLAZA 5.0 server (<https://bioinformatics.psb.ugent.be/plaza/>). All the 12 genes were searched individually. All the information obtained about the gene ID, number of genes, etc were enlisted and compiled systematically. The genes found in the potato genome sequence database were then analysed for their chromosome location and size.

### **Physical mapping of the carotenoids biosynthesis genes and analysis of gene structures**

Locations of the genes were drawn using chromosomal coordinates as per the information given in literature (Genome sequence and analysis of the tuber crop potato (The potato genome sequencing consortium. 2011. *Nature*. 475:189-195). Analysis of genes' structure, introns localization, ORF retrieval etc. were performed using PLAZA 5.0 portal.

### **Estimation of secondary structure, signal peptide and transmembrane regions**

Estimation of secondary structure (for % helix, beta- sheets and coils), signal peptide and transmembrane regions of proteins were performed as per Montgomerie *et al.*, (2008).

### **Alignment of genes, proteins, computing isoelectric point and molecular weight**

Pair-wise/ multiple alignments were performed using Clustal Omega sequence alignment program (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). For computing isoelectric point and molecular weight of various protein, tools available on Expasy resource portal of the SIB Swiss Institute of Bioinformatics (<https://www.expasy.org/>) were used.

## **RESULTS AND DISCUSSION**

### **Genes of carotenoids biosynthesis are present in a range of isoforms and scattered in all the 12 chromosomes except chromosome 9 of potato genome.**

All the twelve genes (presented in Fig.1) viz: GGPS, PSY, ZDS, CRTISO, LCY- $\epsilon$ , LCY- $\beta$ , CHY- $\beta$ ; VDE, ZEP, NXS, CCD and NCED were searched in the potato genome database for their number of gene isoforms and physical location on the potato genome i.e. chromosomes. Six genes each of PSY (IDs: (PGSC0003DMG400021926, PGSC0003DMG400016721, PGSC0003DMG400024063, PGSC0003DMG400029005, PGSC0003DMG400012507, PGSC0003DMG400019372) and CCD (PGSC0003DMG400026032, PGSC0003DMG400018480, PGSC0003DMG400018481, PGSC0003DMG401001968, PGSC0003DMG402001968, PGSC0003DMG400001969, and were found in the data base (Table 1). Five, four and three genes of NCED (PGSC0003DMG400019162, PGSC0003DMG400027633, PGSC0003DMG400015100, PGSC0003DMG400004311, PGSC0003DMG400004312), NXS (PGSC0003DMG400024184, PGSC0003DMG401026407, PGSC0003DMG402019589, PGSC0003DMG400005886) and VDE (PGSC0003DMG400010688, PGSC0003DMG400010690, PGSC0003DMG400020993), respectively, were found in the potato genome sequence database (Table 2). CHY- $\beta$  (PGSC0003DMG400010169, PGSC0003DMG400028897), LCY- $\beta$  (PGSC0003DMG400010637, PGSC0003DMG400008159), GGPS (PGSC0003DMG400027856, PGSC0003DMG400015673)

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showed two copies each. For rest of the four genes viz: ZDS (PGSC0003DMG400022473), CRTISO (PGSC0003DMG400028224), LCY- $\epsilon$  (PGSC0003DMG400000333), and ZEP (PGSC0003DMG400004040) single gene was

found in the potato sequence database (Table 2).

The analysis revealed that these 12 genes and their isoforms were scattered throughout

**Table 2: List of genes of carotenoids metabolic pathway in potato; alongwith their sequence id, chromosome location and size.**

S. No.	Gene	Sequence ID	Chromosome Number	Start Position	End position	Size (basepair)
1	Phytoene synthase (PSY)	PGSC0003DMG400021926	chr02	11294456	11297566	3111
		PGSC0003DMG400016721	chr02	36955673	36959715	4043
		PGSC0003DMG400024063	chr03	2740455	2745217	4763
		PGSC0003DMG400029005	chr04	18612745	18618523	5779
		PGSC0003DMG400012507	chr08	458271	462864	4594
		PGSC0003DMG400019372	chr12	19128104	19129633	1530
2	Beta-carotene hydroxylase (CHY- $\beta$ )	PGSC0003DMG400010169	chr03	43936158	43938595	2438
		PGSC0003DMG400028897	chr06	37867498	37869654	2157
3	Zeta-carotene desaturase (ZDS)	PGSC0003DMG400022473	chr01	75477194	75485576	8383
4	Carotenoid isomerase (CRTISO)	PGSC0003DMG400028224	chr10	56306412	56312609	6198
5	Lycopene beta cyclase (LCY- $\beta$ )	PGSC0003DMG400010637	chr04	12331538	12333464	1927
		PGSC0003DMG400008159	chr10	58302687	58304848	2162
6	Lycopene epsilon cyclase (LCY- $\epsilon$ )	PGSC0003DMG400000333	chr12	3547170	3553905	6736
7	Violaxanthin de-epoxidase (VDE)	PGSC0003DMG400010688	chr04	45916315	45921142	4828
		PGSC0003DMG400010690	chr04	46040209	46044982	4774
		PGSC0003DMG400020993	chr04	46104170	46106015	1846
8	Zeaxanthin epoxidase (ZEP)	PGSC0003DMG400004040	chr02	43804709	43809973	5265
9	Neoxanthin synthase (NXS)	PGSC0003DMG400024184	chr02	21954500	21958056	3557
		PGSC0003DMG401026407	chr02	41342717	41345874	3158
		PGSC0003DMG402019589	chr03	4736917	4745415	8499
		PGSC0003DMG400005886	chr06	54774962	54776783	1822
10	9-cis-epoxycarotenoid dioxygenase (NCED)	PGSC0003DMG400019162	chr05	48865165	48866694	1530
		PGSC0003DMG400027633	chr07	51778895	51781175	2281
		PGSC0003DMG400015100	chr08	10998923	11001084	2162
		PGSC0003DMG400004311	chr08	39185413	39188702	3290
11	Carotenoid cleavage dioxygenase (CCD)	PGSC0003DMG400026032	chr01	69869750	69873902	4153
		PGSC0003DMG400018480	chr08	3615970	3618462	2493
		PGSC0003DMG400018481	chr08	3626959	3631890	4932
		PGSC0003DMG401001968	chr08	47359253	47362257	3005
		PGSC0003DMG402001968	chr08	47360292	47362256	1965
		PGSC0003DMG400001969	chr08	47375775	47378534	2760
12	Geranylgeranyl pyrophosphate synthase (GGPS)	PGSC0003DMG400027856	chr04	69344146	69345467	1322
		PGSC0003DMG400015673	chr11	1682159	1683721	1563

the potato genome, except on chromosome 9 (Fig. 2, Table 2). Maximum numbers of genes/gene-isoforms were found to be present on chromosome 8, and mostly clustered at the ends of the chromosome. Chromosomes 5, 7 and 11 contained only one gene each, whereas, chromosomes 1, 6, 10 and 12 possessed 2 genes each. These genes were found to be varying size and having a wide range of Open reading frames (ORF) and thus encoding proteins differing in number of amino acids (Table 3). Further, the encoded proteins

differed in their calculated molecular weights and isoelectric points (Table 3). Knowledge about the precise physical location of gene(s) on genome is vital to understand the segregation behavior and probability of their crossing & recombination patterns. Genome-wide analysis of a complex pathway like that of carotenoids may assist in devising proper breeding programs for improving total/targeted carotenoid compound(s) in potato.

Having genome-wide information of a gene of gene families is vital for systematic



Fig. 2: Genome-wide landscape of genes of carotenoids metabolic pathway in potato

understanding the role of the gene (s) and their further utilization for improving target trait in plants. For example, Valcarcel *et al.*, (2016) found no relationship between transcript levels of phytoene synthase (PSY) gene and total carotenoid accumulation in potato. The

**Table 3: Structural features (number of introns, open reading frame size) of carotenoid pathway genes and characteristics of the deduced proteins (number of amino acids, molecular weight, theoretical isoelectric point) of carotenoids metabolic pathway in potato.**

Gene	Sequence ID	Number of introns	Open reading frame (nucleotides)	Number of amino acids in deduced protein	Theoretical Isoelectric point/ molecular weight (Dalton)
Phytoene synthase (PSY)	PGSC0003DMG400021926	3	480	159	9.00 / 18404.59
	PGSC0003DMG400016721	5	1317	438	8.76 / 49415.49
	PGSC0003DMG400024063	5	1239	412	8.44 / 46484.11
	PGSC0003DMG400029005	4	681	226	4.84 / 25548.35
	PGSC0003DMG400012507	0	912	303	9.10 / 33727.67
	PGSC0003DMG400019372	2	462	153	4.22 / 17438.83
Beta-carotene hydroxylase (CHY-β)	PGSC0003DMG400010169	6	945	314	9.12 / 35067.69
	PGSC0003DMG400028897	6	930	309	9.30 / 34305.64
Zeta-carotene desaturase (ZDS)	PGSC0003DMG400022473	13	1767	588	8.60 / 64699.45
Carotenoid isomerase (CRTISO)	PGSC0003DMG400028224	12	1848	615	6.92 / 67516.46
Lycopene beta cyclase (LCY-β)	PGSC0003DMG400010637	0	1503	500	6.84 / 56097.91
	PGSC0003DMG400008159	0	1503	500	7.15 / 56336.39
Lycopene epsilon cyclase (LCY-ε)	PGSC0003DMG400000333	2	531	176	5.03 / 19960.65
Violaxanthin de-epoxidase (VDE)	PGSC0003DMG400010688	4	1437	478	6.22 / 54427.16
	PGSC0003DMG400010690	2	927	308	8.57 / 35309.31
	PGSC0003DMG400020993	3	423	140	4.82 / 15702.77
Zeaxanthin epoxidase (ZEP)	PGSC0003DMG400004040	15	2010	669	6.27 / 73025.23
Neoxanthin synthase (NXS)	PGSC0003DMG400024184	4	705	234	9.77 / 25860.44
	PGSC0003DMG401026407	5	750	249	9.60 / 27788.67
	PGSC0003DMG402019589	5	681	226	9.93 / 25513.05
	PGSC0003DMG400005886	0	1497	498	9.13 / 56490.70
9-cis-epoxycarotenoid dioxygenase (NCED)	PGSC0003DMG400019162	0	1530	509	5.96 / 56996.91
	PGSC0003DMG400027633	0	1521	506	5.48 / 56493.34
	PGSC0003DMG400015100	0	1743	580	6.12 / 64708.54
	PGSC0003DMG400004311	12	1824	607	5.80 / 69005.68
	PGSC0003DMG400004312	12	1842	613	6.17 / 69220.04
Carotenoid cleavage dioxygenase (CCD)	PGSC0003DMG400026032	6	1938	645	6.61 / 72869.07
	PGSC0003DMG400018480	2	1143	380	6.11 / 42748.44
	PGSC0003DMG400018481	2	1728	575	6.53 / 64702.59
	PGSC0003DMG401001968	1	723	240	5.82 / 26862.87
	PGSC0003DMG402001968	0	1029	342	8.71 / 38025.08
	PGSC0003DMG400001969	1	1776	591	6.04 / 65237.43
Geranylgeranyl pyrophosphate synthase (GGPS)	PGSC0003DMG400027856	0	1128	375	5.20 / 40657.93
	PGSC0003DMG400015673	0	1098	365	6.38 / 39977.10

probable reason for this lack of correlation might be the analysis of only one isoform of PSY and it might be possible that expression behavior of other isoform(s) of potato might be associated with carotenoid content.

### Carotenoid biosynthesis genes exhibit variations in their genomic structures.

These genes of carotenoid biosynthesis of potato when analyzed for their genomic structures revealed variation in their isoforms. ZEP possessed highest i.e. 15 number of introns. ZDS and CRTISO contained 13 and 12 introns, respectively (Fig. 3, Table 2). Interestingly, of the five genes of NCED, PGSC0003DMG400004311 and PGSC0003DMG400004312 genes contained 12 introns each, whereas remaining three genes did not possess any introns. Both the genes LCY- $\beta$  and GGPS each, lacked the introns (Fig. 3, Table 2). Number of introns in five phytoene synthase gene isoforms varied from 0 to 5. The presence and absence of introns and their numbers in a gene are reported to play important roles in regulating their expression in response to various intrinsic factors (related to growth and development) and external factors. Information about presence/absence of *cis- elements* such as introns is vital to understand the transcriptional behaviour of genes. This is because of the reason that in many eukaryotes, including mammals, plants, yeast, and insects, introns are reported to increase gene expression through a phenomenon termed 'intron-mediated enhancement' (Akua and Shaul, 2013; Shaul, 2017). It has been suggested that introns can increase transcript levels by affecting the rate of transcription, nuclear export, transcript stability and also can increase the efficiency of mRNA translation (Shaul, 2017). Further, the protein encoded by these genes were analysed for their secondary structure composition, and for the presence/absence of

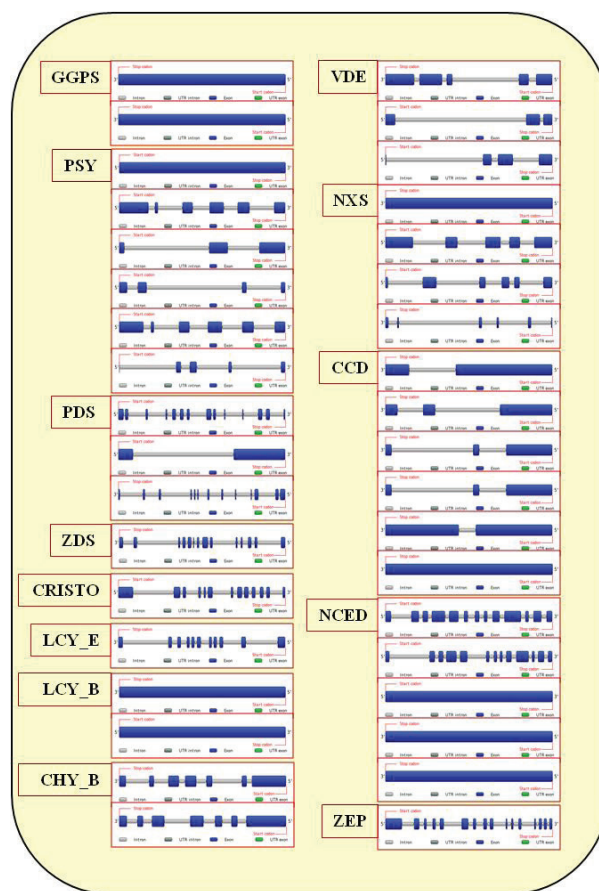


Fig.3: Structure of carotenoids biosynthesis pathway genes of potato (- exon; -intron)

signal peptide and transmembrane domain. Different protein isoforms of these 12 genes differed greatly in terms of their secondary structure composition, signal peptide and transmembrane region. PSY genes encoded six proteins contained 53-71%, 0-8%, and 29-42% helix, beta sheet and random coil secondary structures, respectively (Table 3). Of the six PSY proteins, two were found to have the transmembrane regions. Similarly, CCD genes encoded six proteins contained 2-10%, 30-44%, and 53-62% helix, beta sheet and random coil secondary structures, respectively (Table 4). NCED encoded five proteins did not exhibit much variations in their secondary structure composition. NXS genes encoded four proteins contained 34-

64%, 5-25 %, and 24-42 % helix, beta sheet and random coil secondary structures, respectively (Table 4). Two of the six, PSY proteins (PGSC0003DMG400021926, PGSC0003DMG400019372), both CHY- $\beta$  proteins (PGSC0003DMG400010169 and PGSC0003DMG400028897) and three of the

**Table 4: Predicted secondary structure, signal peptide and transmembrane content of carotenoid pathway genes encoded proteins of potato (<http://www.proteus2.ca/proteus2/index.jsp>)**

Gene	Sequence ID	Secondary structure (%)			Signal Peptide (%)	Transmembrane (%)
		Helix	Beta Sheet	Random Coil		
Phytoene synthase (PSY)	PGSC0003DMG400021926	53	6	42	0	14
	PGSC0003DMG400016721	58	2	39	0	0
	PGSC0003DMG400024063	59	2	39	0	0
	PGSC0003DMG400029005	71	0	29	0	0
	PGSC0003DMG400012507	71	0	29	0	0
	PGSC0003DMG400019372	71	0	29	0	15
Beta-carotene hydroxylase (CHY- $\beta$ )	PGSC0003DMG400010169	55	6	39	3	20
	PGSC0003DMG400028897	53	8	39	0	15
Zeta-carotene desaturase (ZDS)	PGSC0003DMG400022473	31	19	50	0	0
Carotenoid isomerase (CRTISO)	PGSC0003DMG400028224	38	18	44	0	0
Lycopene beta cyclase (LCY- $\beta$ )	PGSC0003DMG400010637	30	23	46	0	0
	PGSC0003DMG400008159	31	24	45	0	0
Lycopene epsilon cyclase (LCY- $\epsilon$ )	PGSC0003DMG400000333	51	8	41	0	0
Violaxanthin de-epoxidase (VDE)	PGSC0003DMG400010688	39	19	42	0	0
	PGSC0003DMG400010690	20	19	61	0	0
	PGSC0003DMG400020993	6	33	61	0	0
Zeaxanthin epoxidase (ZEP)	PGSC0003DMG400004040	25	21	54	0	0
Neoxanthin synthase (NXS)	PGSC0003DMG400024184	58	10	32	0	29
	PGSC0003DMG401026407	64	12	24	0	37
	PGSC0003DMG402019589	56	5	39	0	29
	PGSC0003DMG400005886	34	25	42	0	0
9-cis-epoxycarotenoid dioxygenase (NCED)	PGSC0003DMG400019162	6	39	55	0	0
	PGSC0003DMG400027633	7	37	57	0	0
	PGSC0003DMG400015100	6	38	56	0	0
	PGSC0003DMG400004311	9	35	57	0	0
	PGSC0003DMG400004312	7	34	59	0	0
Carotenoid cleavage dioxygenase (CCD)	PGSC0003DMG400026032	7	31	62	0	0
	PGSC0003DMG400018480	3	44	53	0	0
	PGSC0003DMG400018481	8	37	55	0	0
	PGSC0003DMG401001968	2	42	56	0	0
	PGSC0003DMG402001968	10	30	60	0	0
	PGSC0003DMG400001969	5	35	60	0	0
Geranylgeranyl pyrophosphate synthase (GGPS)	PGSC0003DMG400027856	61	0	39	0	0
	PGSC0003DMG400015673	61	2	36	0	0

four NXS proteins (PGSC0003DMG400024184, P G S C 0 0 0 3 D M G 4 0 1 0 2 6 4 0 7 , PGSC0003DMG402019589) were found to possess transmembrane regions (Table 4). Except for one of the proteins encoded by CHY- $\beta$  gene (PGSC0003DMG400010169) all of the proteins encoded by 12 genes of carotenoid biosynthesis pathway were found to be devoid of signal peptides (Table 3). Sub-cellular localisation of the gene(s) in the cell also has been shown to affect accumulation of corresponding metabolites (Pasare *et al.*, 2013), hence, information about the signal peptide in a gene indicates the probable location of the protein/ enzyme that would be encoded by that gene.

### Phytoene desaturase is present as phytoene dehydrogenase

When the potato genome sequence database was searched for phytoene desaturase gene, strangely, it showed no gene/entry. However, searching potato phytoene desaturase gene from other databases such as NCBI GenBank revealed presence of partially cloned Phytoene desaturase gene (GenBank accession number AY484445) of potato (Supplementary Fig. S1). Keeping in view this interesting and exceptional finding (lack of phytoene desaturase gene in potato genome sequence database), the partially cloned Phytoene desaturase gene available in NCBI Genbank was Blast searched for sequence similarity. Surprisingly, it was observed that the searched potato Phytoene desaturase gene showed highest per cent sequence similarity with that of phytoene dehydrogenase gene of potato (Fig. 3). This was further confirmed when proteins encoded by these two genes were searched for presence of the conserved domains. Both these proteins possessed phytoene desaturase conserved domain (Fig.4). Based on this finding, potato genome sequence database was then searched for phytoene

>AY484445.1 *Solanum tuberosum* phytoene desaturase (pds) mRNA, partial cds; nuclear gene for chromoplast product  
 TTTCAGTAAAATGCCTCAAATGGACTGTGTTCTGTGCTGTAATTTGAGAGTCCAAGGTAGTTCAGCTTAT  
 CTTTGAGCTCGAGGTGCTCTTTGGGAACGAAAGTCGAGATGGTTGCTGCAAAAGGAATTCGTTAT  
 GTTTGCTGTGACGAATCAATGGGTCATAAGTTAAAGATTCTGCCACGACCAGAAGATTGGTTAAGGA  
 CTTGGGGCTTTAAAGGTAGTAGTCATTGATTATCCAAGCAGACGACTAGACAATAACAGTTAACTATTGG  
 GAGGCTGATTCTTATCATCAACATTCGGTCTTCCCGCCCACTAAACCATTTGGAGATTGTTATTG  
 CTGTGCGAGTTGGTGGTTGTCTACAGCAAAAATTTGGCAGATGCTGGTGCACAAACCCGACTACT  
 GGAGGCAAGGATGTTCTAGGTGGAAGGTAGTCATGGAAGATGATGATGGAGATTGGTACGAGACT  
 GGTTGTCATATATTCTTTGGGCTTACCCAAATATTCAGAACCTGTTGGAGAATTAGGGATTAATGATC  
 GATTGCAAGGAAACATTCATGATATTGCAATGCCAAGTAAGCCAGGAGAATTTAGCCGCTTGA  
 TTTCCCGAAGCTTTACCCGCTCTTTAAATGGAGTTTGGCCATCTAAAGAACAATGAAATGCTTACA  
 TGGCCAGAGAAAGTCAAATTTGCAATGGACTCTGGCAGCAATGCTGGAGGCAATCTTATGTTGAAG  
 CTCAAAGACGGATAAGTGTTAAGGACTGGATGAGAAAGCAAGGTGTCGGGATAGGGTGACAGATGAAT  
 GTTCATCGGCATGTCAAAGGCACTTAACCTTATAAACCCCTGACGAACCTGTCAATGCAAGTGCATCTGATC  
 CAGTTGAACAGGTTTCTCCAGGAGAAACATGGTTCAAAAATGGCTTTTATGATGGTAATCTCTCGAGA  
 GACTTTGCATCCGATGTTGAACACATCGAGTCAAAGGTGGCCAAAGTCAGATTGAACACAGCAATAAA  
 AAAGATTGAGTTGAATGAGGATGGGAGTCTCAAGTGTTTTAACTGAATGACGGTAGTACAGTTGAGGGC  
 GATGCTTTTGTGTTGCACTCCAGTGGATATTTCAAGCTGCTTTGCCCTGAAGACTGGAAAGAGATT  
 CATATTTCCAAAAGTTGGAGAAGTTAGTCGGAGTACCTGTTATAAATGTACATATATGTTTCAGACAAA  
 ACTGAAGACACATATGATCATTTGCTCTCAGCAGAAAGCTCAGTCTCAGTGTGATGCTGACATGCT  
 GTCACATGTAAGGAATATTACAACCCCAATCAGTCTATGTTGAATGGTTTTTGGCACTGCAGAAAGAT  
 GGATATCTCGCAGGACTCAGAAAATAATTGATGCTCAGATGAAGAACTAGCAACACTTTTCTGATGA  
 AATTTGACAGATCAAAGCAAGCAAAAATATAAAGATTCATGTTGCAAAAACCTCAAGGTCTGTTTAT  
 AAAACAGTGCCAGGTGTGAACCTGTGCGCCATGCAAAAGATCCCTATAGAGGGGTTTTATTAGCCG  
 GTGA

Supplementary Fig. S1: Partially cloned phytoene desaturase gene from *Solanum tuberosum* available in NCBI GenBank (accessed on March 2023)

dehydrogenase gene, which revealed presence of three phytoene dehydrogenase genes viz: PGSC0003DMG400009156, P G S C 0 0 0 3 D M G 4 0 0 2 6 5 5 0 , and PGSC0003DMG402003505. Of the three genes, PGSC0003DMG400009156 was found to be located on chromosome 3 and other two on chromosome 2 (Table 5). The sizes of these three genes were found to be ranging from 868 to 8921 basepair. Thirteen, one and twelve introns were found to be present in PGSC0003DMG400009156, P G S C 0 0 0 3 D M G 4 0 0 2 6 5 5 0 , and PGSC0003DMG402003505 genes respectively (Fig. 5), and possessing ORF of 1752, 345, and 1398 nucleotides, respectively (Table 5). Phytoene dehydrogenase genes encoded three proteins contained 15-48 %, 0-92 %, and 19-43 % helix, beta sheet and random coil secondary structures, respectively. All these three phytoene dehydrogenase proteins lacked signal peptide sequence and transmembrane regions (Table 5). The result of this study suggests that thorough analysis of genes is important before concluding absence of a gene in a plant.

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KJ-ZEP PGSC0003DMG400004020	ATGATTTCAACTGTGTTTACTTCTTCAGTCACTCCCTCCACTCAGTTTTTCAAGAAAG ATGATTTCAACTGTGTTTACTTCTTCAGTCACTCCCTCCACTCAGTTTTTCAAGAAAG	60 60	AY484445_PSY XM006342818_FHM	ATGCCCTAAATGGACTTGTCTGCTGTTAATTTGAGAGTCCAGATGATGACTGTAT	60 0
KJ-ZEP PGSC0003DMG400004020	CACCGCCCTTATGATTTCCAGAGCCTTCCGACAGATATATATCATCTATACCTGTC CAGCTACCTTATGATTTCCAGAGCCTTCCGACAGATATATATCATCTATACCTGTC	120 120	AY484445_PSY XM006342818_FHM	CTTTGGAGCTCGAGTGTCTCTTTGGGAACTGAAAGTCGAGATGTTCTGCAAGG -----CGAGTGTCTCTTTGGGAACTGAAAGTCGAGATGTTCTGCAAGG	120 150
KJ-ZEP PGSC0003DMG400004020	AGGAGCTTGGAAATGGCCATATCAGAAAGTTAAAGAGTAAAGTAAAGCCACATA AGGAGCTTGGAAATGGCCATATCAGAAAGTTAAAGAGTAAAGTAAAGCCACATA	180 180	AY484445_PSY XM006342818_FHM	AATTCGTATGTTTTGCTGGTAGGAAATCAATGGTTCATAAGTTAAAGATCGT----- AATTCGTATGTTTTGCTGGTAGGAAATCAATGGTTCATAAGTTAAAGATCGTACTCCC	174 110
KJ-ZEP PGSC0003DMG400004020	GCTGAAGCTCCGGTACTCTACAGAGAAGAACTCTGGGGTAAAGGATTAAGG ACTGAAGCTCCGGTACTCTACAGAGAAGAACTCTGGGGTAAAGGATTAAGG	240 240	AY484445_PSY XM006342818_FHM	-----GCCACACAGAAAGTGGTAAAGACTGGGGCTTTAAAGTAGTATGCTGATG CATGCCACACAGAAAGTGGTAAAGACTGGGGCTTTAAAGTAGTATGCTGATG	231 170
KJ-ZEP PGSC0003DMG400004020	GTTCACAGAAAGTGAAGTACTTCTCCCGGTGGGATGGAGGTTAGTTTTT GTTCACAGAAAGTGAAGTACTTCTCCCGGTGGGATGGAGGTTAGTTTTT	300 300	AY484445_PSY XM006342818_FHM	TATCCAGACCCAGAGCTAGACATACAGTAACTATTGGAGGCTCATCTTATCATCA TATCCAGACCCAGAGCTAGACATACAGTAACTATTGGAGGCTCATCTTATCATCA	291 230
KJ-ZEP PGSC0003DMG400004020	GCTTACAGCAAAAGAAAGGGTGTATGTTGTGTGTGGAGGATTAAGTGTCT GCTTACAGCAAAAGAAAGGGTGTATGTTGTGTGTGGAGGATTAAGTGTCT	360 360	AY484445_PSY XM006342818_FHM	ACATTCCTGTCTCTCCGCGCCAACTAAACATTTGAGATGTTATTGCTGGTCAGGT ACATTCCTGTCTCTCCGCGCCAACTAAACATTTGAGATGTTATTGCTGGTCAGGT	351 290
KJ-ZEP PGSC0003DMG400004020	ATCAGAGAGAGGCAATATAGAGTCCATTCAGATACAGAGCATCATTGGCTGCT ATCAGAGAGAGGCAATATAGAGTCCATTCAGATACAGAGCATCATTGGCTGCT	420 420	AY484445_PSY XM006342818_FHM	TTGGGTGTTTGTCTACAGAAAATTGGCAGATCTGGTCACACAGCATACTACTG TTGGGTGTTTGTCTACAGAAAATTGGCAGATCTGGTCACACAGCATACTACTG	411 350
KJ-ZEP PGSC0003DMG400004020	TTGGAAGCAATGATATGATGTTGCTGAAGCATCATGAATGCTGCTCATCACTGGT TTGGAAGCAATGATATGATGTTGCTGAAGCATCATGAATGCTGCTCATCACTGGT	480 480	AY484445_PSY XM006342818_FHM	GAGCCAGGGATTTCTAGTGGAAAGTAGTCTGCTGAAAGATGATGAGGATTGG GAGCCAGGGATTTCTAGTGGAAAGTAGTCTGCTGAAAGATGATGAGGATTGG	471 410
KJ-ZEP PGSC0003DMG400004020	CAAGAGTAAAGGCTTGGTGTATGTTTCTGGCACTGATTAAGGATTTGATAGC CAAGAGTAAAGGCTTGGTGTATGTTTCTGGCACTGATTAAGGATTTGATAGC	540 540	AY484445_PSY XM006342818_FHM	TACGAGCTGGTTGCATATATTCTTGGGCTTACCCAAATATTCAGAACCTGTTTGA TACGAGCTGGTTGCATATATTCTTGGGCTTACCCAAATATTCAGAACCTGTTTGA	531 470
KJ-ZEP PGSC0003DMG400004020	TTCACTCCAGAGTGAAGTGTGCTCCCGTCAAGAGCATCAGCCGATGACTTGG TTCACTCCAGAGTGAAGTGTGCTCCCGTCAAGAGCATCAGCCGATGACTTGG	600 600	AY484445_PSY XM006342818_FHM	GAATTAGGATTAATGATGATTCGATGGAGAACTCAATGATATTGCAATGCCA GAATTAGGATTAATGATGATTCGATGGAGAACTCAATGATATTGCAATGCCA	591 530
KJ-ZEP PGSC0003DMG400004020	CAGCAGTCTTCCACTGCTGTGGGAGATACAAATTAATGAAATGATTTGTTGA CAGCAGTCTTCCACTGCTGTGGGAGATACAAATTAATGAAATGATTTGTTGA	660 660	AY484445_PSY XM006342818_FHM	AGTAAGCAGAGAAATTTAGCGCTTTGATTTCCCGAAGCTTTACCGCTCTTAAAT AGTAAGCAGAGAAATTTAGCGCTTTGATTTCCCGAAGCTTTACCGCTCTTAAAT	651 590
KJ-ZEP PGSC0003DMG400004020	GACTTTGAGAGTGGGAGAGGTTAGTGTGCTTCTTGAAGATGACACCAATTACA GACTTTGAGAGTGGGAGAGGTTAGTGTGCTTCTTGAAGATGACACCAATTACA	720 720	AY484445_PSY XM006342818_FHM	GGAGTTTGGCACTTCAAGAACAAAGAAAGTCTTACATGCGCAGAGAACTCAATTT GGAGTTTGGCACTTCAAGAACAAAGAAAGTCTTACATGCGCAGAGAACTCAATTT	611 650
KJ-ZEP PGSC0003DMG400004020	GGTGATCTCTGCTGGTGTGATGATGATGCTTAAGTACGCAATATTTGTTTGA GGTGATCTCTGCTGGTGTGATGATGATGCTTAAGTACGCAATATTTGTTTGA	780 780	AY484445_PSY XM006342818_FHM	GCAATTGGACTCTGGCAGCAATGCTGGAGGGCACTTATGTTGAGCTCAAGCGGG GCAATTGGACTCTGGCAGCAATGCTGGAGGGCACTTATGTTGAGCTCAAGCGGG	771 710
KJ-ZEP PGSC0003DMG400004020	CCGAGTAGTACTTCTGCTGCTACTGTTTACTGCAATGAGATTTGTTTCT CCGAGTAGTACTTCTGCTGCTACTGTTTACTGCAATGAGATTTGTTTCT	840 840	AY484445_PSY XM006342818_FHM	ATAAGTGTAAAGCTGGATGAGAAAGAGGTTGCTCCGAGAGGGTACAGATGAAGTG ATAAGTGTAAAGCTGGATGAGAAAGAGGTTGCTCCGAGAGGGTACAGATGAAGTG	831 770
KJ-ZEP PGSC0003DMG400004020	GTGATCTCCAAAGTGAAGAAAGGATGCTTAAATATTGGGGATGGTGTGAC GTGATCTCCAAAGTGAAGAAAGGATGCTTAAATATTGGGGATGGTGTGAC	900 900	AY484445_PSY XM006342818_FHM	TTCACTGGCACTCAAGGCACTTAACTTAAACCTGACGAACTGCAATGCAATGCT TTCACTGGCACTCAAGGCACTTAACTTAAACCTGACGAACTGCAATGCAATGCT	891 830
KJ-ZEP PGSC0003DMG400004020	AATGTCATAGACCTTATGTTCCACAGATGAAGTCAATTTCTGCTGACATCAT AATGTCATAGACCTTATGTTCCACAGATGAAGTCAATTTCTGCTGACATCAT	1080 1080	AY484445_PSY XM006342818_FHM	ATCTTGATGCTTGAAGAGTCTTCCAGAGAACTGGTCAAATAGGCTTTTFTA ATCTTGATGCTTGAAGAGTCTTCCAGAGAACTGGTCAAATAGGCTTTTFTA	951 890
KJ-ZEP PGSC0003DMG400004020	GATAGACCAACAACTTTAGTGGGAGAGGTCATGTTAGATGCTTGGGACTCTGTC GATAGACCAACAACTTTAGTGGGAGAGGTCATGTTAGATGCTTGGGACTCTGTC	1140 1140	AY484445_PSY XM006342818_FHM	GGGTAACCTCTGAGAGACTTGCATGGCTGTTTGAACACAGCGATCAAAAGGT GGGTAACCTCTGAGAGACTTGCATGGCTGTTTGAACACAGCGATCAAAAGGT	1011 950
KJ-ZEP PGSC0003DMG400004020	CATGCTATGACCTTAAATTTGGGTCAAGGGGATCATGGCTAGAGATGATCAACA CATGCTATGACCTTAAATTTGGGTCAAGGGGATCATGGCTAGAGATGATCAACA	1200 1200	AY484445_PSY XM006342818_FHM	GGCCAGTCAAGTGAACCTCAAGAAATAAAGATGATGATGATGATGATGATGCTC GGCCAGTCAAGTGAACCTCAAGAAATAAAGATGATGATGATGATGATGATGCTC	1071 1010
KJ-ZEP PGSC0003DMG400004020	CTAGCAGCTGACAGAGAGTATGATGATGATGATGATGATGATGATGATGATGAT CTAGCAGCTGACAGAGAGTATGATGATGATGATGATGATGATGATGATGATGAT	1260 1260	AY484445_PSY XM006342818_FHM	ANGTGTTTATAGTGAATGAGGTAGTACAGTGGAGGGGATGCTTTGTTGTTCCACT ANGTGTTTATAGTGAATGAGGTAGTACAGTGGAGGGGATGCTTTGTTGTTCCACT	1131 1070
KJ-ZEP PGSC0003DMG400004020	ATCATCTCATCTTTAAGAGCTATGAAGTCTGAGAAATTCGAGTGGAGTCAATCAT ATCATCTCATCTTTAAGAGCTATGAAGTCTGAGAAATTCGAGTGGAGTCAATCAT	1320 1320	AY484445_PSY XM006342818_FHM	CCAGTGGATATTTCAAGCTGCTTTGCTGAGAGCTGGAAGAGATCCATATTCCAA CCAGTGGATATTTCAAGCTGCTTTGCTGAGAGCTGGAAGAGATCCATATTCCAA	1191 1130
KJ-ZEP PGSC0003DMG400004020	GGACTGGAGATGGCTCAATCATGCACTACTTCAAAAGCTTACTTGGGCTCGGA GGACTGGAGATGGCTCAATCATGCACTACTTCAAAAGCTTACTTGGGCTCGGA	1380 1380	AY484445_PSY XM006342818_FHM	ANGTGGAGAGTGTGCTGAGACTCTGATTAATGATCAATGATGATGATGATGATGAT ANGTGGAGAGTGTGCTGAGACTCTGATTAATGATCAATGATGATGATGATGATGAT	1251 1190
KJ-ZEP PGSC0003DMG400004020	CTAGCAGCTGACAGAGAGTATGATGATGATGATGATGATGATGATGATGATGAT CTAGCAGCTGACAGAGAGTATGATGATGATGATGATGATGATGATGATGATGAT	1440 1440	AY484445_PSY XM006342818_FHM	CTGAGAACACATATGATCATTGCTCTTCCAGAGAGCTCACTGCTCAGTGTATGCT CTGAGAACACATATGATCATTGCTCTTCCAGAGAGCTCACTGCTCAGTGTATGCT	1311 1250
KJ-ZEP PGSC0003DMG400004020	AGATATTTTGAATGGAGAGGCTCTGATGATGATGATGATGATGATGATGATGAT AGATATTTTGAATGGAGAGGCTCTGATGATGATGATGATGATGATGATGATGAT	1500 1500	AY484445_PSY XM006342818_FHM	GCATCTGCTCAGTCAATGAGGATATTAACACCCCACTGCTGATGATGATGATGATGAT GCATCTGCTCAGTCAATGAGGATATTAACACCCCACTGCTGATGATGATGATGATGAT	1371 1310
KJ-ZEP PGSC0003DMG400004020	GCAAGCTGAGAGAGTAAATCAATGAGCTTACTGAGAAAGCAATGACCAATG GCAAGCTGAGAGAGTAAATCAATGAGCTTACTGAGAAAGCAATGACCAATG	1560 1560	AY484445_PSY XM006342818_FHM	TTTGACCTGAGAGAGTGGATATCTCCAGAGCTCAGAAATTAATGATGATGATGATGAT TTTGACCTGAGAGAGTGGATATCTCCAGAGCTCAGAAATTAATGATGATGATGATGAT	1431 1370
KJ-ZEP PGSC0003DMG400004020	AGAAATGTTGAGAGATGATGATGATGATGATGATGATGATGATGATGATGATGAT AGAAATGTTGAGAGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	1620 1620	AY484445_PSY XM006342818_FHM	AGGAACTAGCAACACTTTTCTGATGAAATTCAGAGAGTCAAGCAAGCAAAATA AGGAACTAGCAACACTTTTCTGATGAAATTCAGAGAGTCAAGCAAGCAAAATA	1491 1430
KJ-ZEP PGSC0003DMG400004020	TTACTGGGGAGAGGACTTCTGGTGAAGAGTATGTTTAAAGCAGATGAGATG TTACTGGGGAGAGGACTTCTGGTGAAGAGTATGTTTAAAGCAGATGAGATG	1680 1680	AY484445_PSY XM006342818_FHM	TTAAATATCATGTTGCTCAAAGCTTAAAGTCTGTTTAAAGCAAGTCCAGGTTGAA TTAAATATCATGTTGCTCAAAGCTTAAAGTCTGTTTAAAGCAAGTCCAGGTTGAA	1551 1490
KJ-ZEP PGSC0003DMG400004020	GTCCCTGACATATGCGGCTCTCTCACACAAATCTCCGAAATCAGTATGTTTA GTCCCTGACATATGCGGCTCTCTCACACAAATCTCCGAAATCAGTATGTTTA	1740 1740	AY484445_PSY XM006342818_FHM	CCTTGGCCACTGCAAGACTCCCTAGAGGGGTTTATTAGCCGGTGA----- CCTTGGCCACTGCAAGACTCCCTAGAGGGGTTTATTAGCCGGTGA-----	1604 1550
KJ-ZEP PGSC0003DMG400004020	CTTTGGCAGAGTCTGAAAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT CTTTGGCAGAGTCTGAAAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT	1800 1800	AY484445_PSY XM006342818_FHM	----- AAACAGAAATACTGGCTCAATGGAGGGCTGCTTATCAGAAAGCTTTGGCGCAA	1604 1610
KJ-ZEP PGSC0003DMG400004020	GTAAGCTGATGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT GTAAGCTGATGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT	1860 1860	AY484445_PSY XM006342818_FHM	GCTATTGTACAGAGTATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT GCTATTGTACAGAGTATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	1604 1670
KJ-ZEP PGSC0003DMG400004020	CGAGACTTCCAAACTTCCCTACAGCTTTCCATCCATCAGATGTTATGAAATTTGGTCT CGAGACTTCCAAACTTCCCTACAGCTTTCCATCCATCAGATGTTATGAAATTTGGTCT	1920 1920	AY484445_PSY XM006342818_FHM	----- AGCTAGTTTAG 1604 AGCTAGTTTAG 1682	1604 1670
KJ-ZEP PGSC0003DMG400004020	GATAAGCTGATGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT GATAAGCTGATGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGAT	1980 1980	AY484445_PSY XM006342818_FHM	----- AGCTAGTTTAG 1604 AGCTAGTTTAG 1682	1604 1670
KJ-ZEP PGSC0003DMG400004020	GAGAGCTGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGATGAT GAGAGCTGAGAGAGTCAAGGCTGATGATGATGATGATGATGATGATGATGATGAT	2040 2040	AY484445_PSY XM006342818_FHM	----- AGCTAGTTTAG 1604 AGCTAGTTTAG 1682	1604 1670

Fig.3: Pair-wise alignment of Phytoene Desaturase gene sequence available on NCBI GenBank (NCBI Accession No. AY484445) with that of Phytoene Dehydrogenase (NCBI Accession No. XM\_006342818) of potato. The Blast analysis of Phytoene Desaturase from potato showed maximum identity (90 %) with that of the Phytoene Dehydrogenase.

Supplementary Fig.S2: Pairwise alignment of nucleotide sequence cloned KJ-ZEP gene cloned from potato with that of available in Potato Genome Sequence Database (PGSC0003DMG400004020).

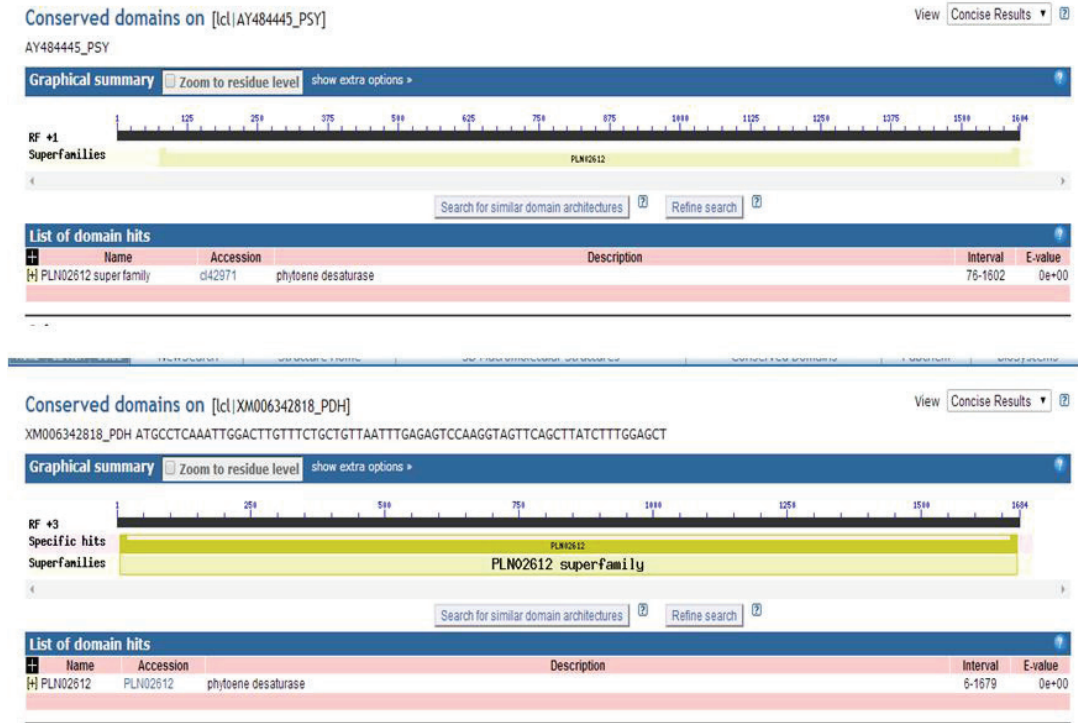


Fig.4: Conserved domain (CD) search analysis of Phytoene Desaturase gene sequence available on NCBI GenBank (NCBI Accession No. AY484445) with that of Phytoene Dehydrogenase (NCBI Accession No. XM\_006342818) of potato. The CD analysis of both these genes showed presence of Phytoene Desaturase domain in both the proteins encoded by these genes and thus conforms that both the genes are similar in sequence, structure and function.

Table 5: Features of Phytoene Dehydrogenase genes of potato

Feature	Phytoene Dehydrogenase genes of potato		
	PGSC0003 DMG400009156	PGSC0003 DMG400026550	PGSC0003 DMG402003505
Chromosome Number	chr03	chr02	chr02
Start Position	61872343	24917817	40493434
End position	61879640	24918684	40502354
Size (basepair)	7298	868	8921
Number of introns	13	1	12
Open reading frame (nucleotides)	1752	345	1398
Number of amino acids in deduced protein	583	114	465
Theoretical Isoelectric point/ molecular weight (Dalton)	6.41 / 64967.98	4.25 / 12878.51	7.01 / 50960.10
Secondary structure			
(i) Helix (%)	37	92	38
(ii) Beta Sheet (%)	15	0	19
(iii) Random Coil (%)	48	8	43
Signal Peptide (%)	0	0	0
Transmembrane (%)	0	0	0

## Zeaxanthin epoxidase gene cloned from Kufri Jyoti cultivar of potato exhibited sequence variations at several positions.

Furthering the work on carotenoid metabolism in potato a *Zeaxanthin epoxidase* (*ZEP*) gene was cloned from Kufri Jyoti cultivar of potato using different set of primers listed in the Table 1. As the size of the cloned genes was more than 2 kilobase pair, and thus, sequencing of the complete gene as such was not possible (the used gene sequencer gives accurate sequencing upto 600 base pair). For this reason, the overlapping fragments were sub-cloned (Fig. 6) and then sequenced in order to get the overlapping sequences required for alignment and for deriving the final complete sequence of the gene. The cloned and sequenced *ZEP* gene from Kufri Jyoti cultivar of potato is hereinafter referred to as KJ-*ZEP*. The KJ-*ZEP* gene sequence was submitted to NCBI GenBank and was assigned NCBI accession number MK852682. The complete ORF of cloned KJ-*ZEP* was 2010 nucleotides long (Fig. 7) and encoding a protein having 669 amino acids (Fig 8). It exhibited variations at 9 amino acid positions when compared with the one available in potato genome sequence database (Fig. 9, Table 6) and at 14 nucleotides (Supplementary Fig S2). Based on these variations the clone KJ-*ZEP* may be considered as new isoform of *ZEP* in potato. These variations at 9 amino acids might provide some uniqueness to the cloned gene which might be involved in regulation of carotenoid biosynthesis pathway especially beta-carotene, violxanthin, zeaxanthin carotenoids etc. Potential of zeaxanthin epoxidase as a target gene for increasing zeaxanthin and total carotenoid content has already been reported in potato (Romer *et al.*, 2002).

## CONCLUSION

Although carotenoid biosynthesis and their regulation have been subjected to extensive

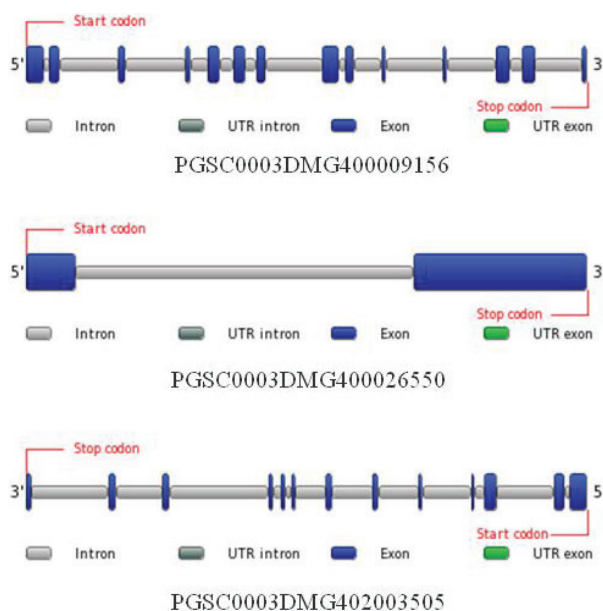


Fig.5: Structure of *Phytoene Dehydrogenase* genes of potato (- exon; -intron).

Table 6: Variations in amino acid sequences of cloned KJ-*ZEP* encoded protein when compared with the one encoded by *ZEP* gene available in Potato Genome Sequence Database (PGSC0003DMG400004020).

S. No.	Position of amino acid	Amino acid present in PGSC0003DMG400004020 encoded protein	Amino acid present in cloned KJ- <i>ZEP</i> gene encoded protein
1	8	Threonine (T)	Serine (S)
2	16	Isoleucine (I)	Valine (V)
3	17	Leucine (L)	Phenylalanine (F)
4	32	Threonine (T)	Alanine (A)
5	38	Leucine (L)	Isoleucine (I)
6	60	Isoleucine (I)	Leucine (L)
7	61	Threonine (T)	Alanine (A)
8	71	Serine (S)	Asparagine
9	643	Alanine (A)	Valine (V)

studies, yet, much remains to be elucidated. Various enzymes have been shown to contribute to the regulation of carotenoids biosynthesis. Despite extensive studies, our understanding of carotenoid metabolism, regulation, and roles of carotenoid derivatives is still developing. A comprehensive understanding of the regulation of carotenoid accumulation

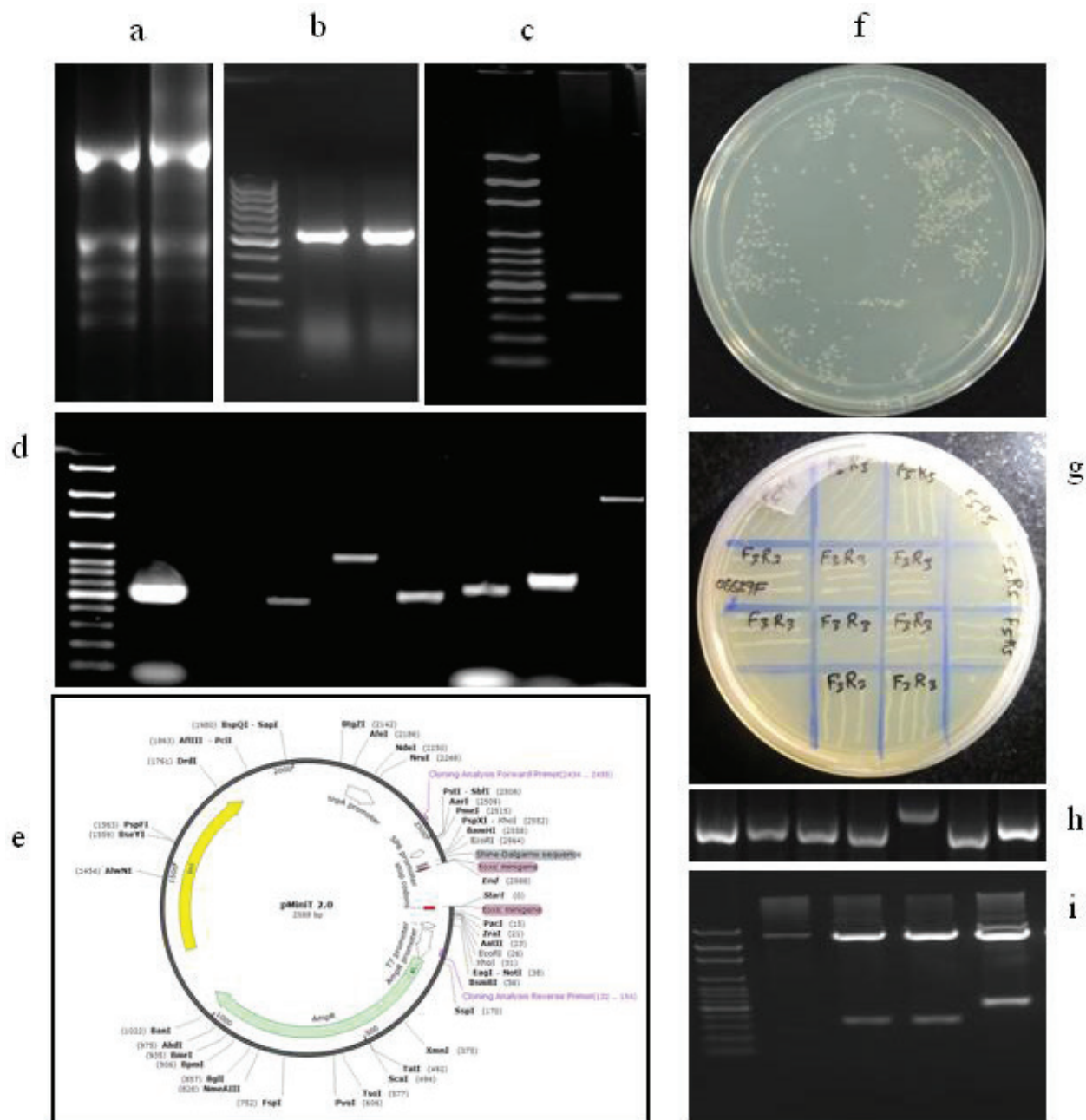


Fig.6: cloning of Zeaxanthin epoxidase (ZEP) from Kufri Jyoti cultivar of Potato. (a) RNA confirmation gel, (b) 26s rRNA confirmation (L1-100 BP ladder, L2 and L3 amplified 26s rRNA). (c): partially amplified gene (L1- 100bp ladder, L2 partially amplified amplicon primer used 06629-F-F2R2). (d): amplicons obtained upon PCR ( L1- 100bp ladder, L2- 26s, L3 -06629-F-F1R1, L4-06629-F-F1R2, L5- 06629-F-F2R2, L6-06629-F3R3,L7 06629-F-F4R4,L8-06629-F-F5R5). (e): Map of vector pMiniT2.0 used in cloning. (f): Transformed colonies of the E.coli on ampicillin plate of ZEP gene.(g): colonies selected from the transformed plate on the selection media/plate. (h): agarose gel electrophoretic analysis of colony PCR. (i) Restriction digestion confirmation of inserted gene (L1 100BP ladder, L2-L5 restriction digestion).

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ATGATCAACTGTTTACTCTTCAGTTCATCCTCCACTCAGTTTTTTCAAGAAAGCA
GCTGCCTTATTGATTTCCAAGGACTTCCGTCAGAGTTATATCATCTATACCTGTAGGA
GCTTGGAAAATGGGCATATCAAGAAGTTAAAGGATAAAAGTAAAGCCACACTAGCTGAA
GCTCCGGTTACTCTACAGAGAAATGACTCTGGGTTAACCGTGATTGAGGTTCCACA
GAAGAAGTTGAAGTACTTGTGCCGGTGGGATTGGAGGGTGTAGTTTTGCTTTAGCAG
CAAAGAAAAGGGGTTTGTATGTGTGGTGTGAGAGGGATTAAAGTCTATCAGAGAGAG
GGCAAAATAGAGGTTCAATTCAGATACAGAGCAATGCATTGGCTGCTTTGGAAGCAATTGA
TATGGATGTTCTGAAGACATCATGAATGCTGGCTGCATCACTGGTCAAAGGATTAATGGCT
TGGTGTATGGTATTTCGGCACTGGTATTGCAAGTTGATACGTTCACTCCAGCAGTGGAA
CGTGGACTCCCGTGACAAGAGTATCAGCCGCATGACTTTGACAGAGATCCTTGACAGTGC
TGTTGGGGAGGATACAAATTATGAATGAAAGTAAATGTTGTAGACTTTGAGGATGATGGGGAGA
AGGTTAGTGTGGTCTTGAGAAATGGAACAACGATTTACAGGTGATCTCTGTGTGGTGTGAT
GGCATAAGGCTTAGAGTACGGACTAATTTGTTGGCCAGCAGTAACTACTTACTCTGGCTA
CACTTGTATACTGGAATTCAGATTTGTTCCCTGCTGATATTGATACAGTGGGTACCGAG
TCTTTTTGGGCCACAACACTACTTTGTTTCTCAGATGTGGGGGAGGCAAGATCAGTGG
TATGCATTTTACAATGAACAGCTGGTGTGTGGATGCTCCAAATGGTAAAAGGAAAGATT
GCTTAAAATATTGGGGATGGTGTGACAATGTCATAGACCTATTAGTTGCCACAGATGAAG
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GTTACATTTGCTGGGACTCTGTCCATGCTATGCAGCCTAATTTGGGTCAAGGGGAGCAT
GGCCATAGAGGATAGTATCAACTAGCAGTGAACCTGACAAGCATGTAGTCGAAGTGGCC
AGTCAGGAAGCCCTGTGGATATCATCTCATCTTAAGGAGCTATGAAAGTGTAGAAAACCT
CGAGTTGGAGTATCCATGGACTGGCTAGAATGGCTGCAATCATGGCATCTACTTACAAGAC
TTATCTTGGCTCGGACTAGTCCACTATCAATTTTGGAGCAGTATAGAATACCACATCTCTG
GAAGAGTTGGTGAAGAGATTTTATTGACTTGGGAATGCCTCTGATGTTAAGTTGGGTTCTA
GGAGCAATGGGCAACAGCTTGAAGCCAGAATAAACAATTCAGGCTATCTGAGAAAACAAA
TGACCAATTGAGAAAATGGTTTGAAGATGATGATGATGATGATGATGATGATGATGATGATG
GGTACTTTTACTTGGGGGAAATGGCACTCTGGTTTGAAGCTATTGTTTAAAGCAGAGAT
GAGGATGCTCTTGCATCTATGGGCTGTGTCACATCAACAACATCTCTGGAATAATCATAGT
TTTACTTTGGCCACAGTGTCTGAATGCAAGCCCAATATCTCTCAAGACCGAGCATTTT
TTGTAATGATTTCAAAGCAACATGGCACCTGGGTTACAGATAATGAGGCGAAGATAC
CGGACGCTCCAACACTTCCCTACACGTTTTCATCCATCAGATGTTATCGAATTTGGTCTGA
TAAGGTAGCATTCTGTTAAGGCAATGAAATTTCTCCAAAACACTGAAAAGGAAAGAA
AGCGTGAAGCAGTGGGGCCAGCAGCTAA
    
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Fig. 7: Complete Open Reading Frame (ORF) of KJ-ZEP gene cloned from potato cultivar Kufri Jyoti.

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MYSTVFYSVHPSTSVFSSRKQLPFLISKDFPFLYHSLICRSLENGHIKKVGVKVALEI
TEKNDSGVNGDLKVPQKLLKVLVAGGGIGGLVFLAALKKGFVLDLVERDLSAIRGEGYRGP
SNAALALEADMDVAEDIMNAGCITGQRINGLVDGISGNWYCKFDFTFAVERGLPVTVRIS
QILARAVGDTIMNESNVDFEDDGEKVSVVLENGQRFITDIDTVGYRVLGHKQYFVS
YTCYTG IADFPVAD IDTVGYRVLGHKQYFVS SDVGGGKMQWYAFVNEPAGGVDAPFHGK
GGWCDNVIDLVADEDAILRRDIYDRPPTFSWGRGHVTLGGDSVHAMQPNLQGGGCHAI
LELDKACRSFAESGSPVDIISLRSYESARKLRVGVIHGLARMAAIMASTYKAYLVGLG
YRIPHPGRVGVFDLGHPLMLSVVLGGNGDKLEGR IKHCRLESEKANDQLRKFEDD
EWLLLPAGNGTSGLEAIVLSRDEDPCTIGSVSHNIPGKSVVLPQVSEMHARISCKDGA
LQSEHGTVWTDNEGRRYRTPNFPTFRFHPSDVIEFGSDKVAFRVKAMKFPKPTTERK
EEREAVGAA
    
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Fig. 8: deduced protein from cloned Zeaxanthin epoxidase (ZEP) from Kufri Jyoti cultivar of Potato (KJ-ZEP)

is critical in providing novel strategies for such endeavour. The present findings about presence of different isoforms of the carotenoids biosynthesis genes in potato and their genome-wide distribution pattern will provide deeper insights into the mechanism of carotenoid synthesis, spatial distribution and assist plant breeders and biotechnologists to devise and apply precision breeding tools for enhancing total carotenoid content or

PGSC0003DMG400004020	MYSTVFYSVHPSTSVFSSRKQLPFLISKDFPFLYHSLICRSLENGHIKKVGVKVALEI	60
KJ-ZEP	MYSTVFYSVHPSTSVFSSRKQLPFLISKDFPFLYHSLICRSLENGHIKKVGVKVALEI	60
PGSC0003DMG400004020	TAAPVPTPEKNSISGVNGDLKVPQKLLKVLVAGGGIGGLVFLAALKKGFVLDLVERDLSA	120
KJ-ZEP	TAAPVPTPEKNSISGVNGDLKVPQKLLKVLVAGGGIGGLVFLAALKKGFVLDLVERDLSA	120
PGSC0003DMG400004020	IRGEGYRGPFIQIQSNALAALEADMDVAEDIMNAGCITGQRINGLVDGISGNWYCKFDFT	180
KJ-ZEP	IRGEGYRGPFIQIQSNALAALEADMDVAEDIMNAGCITGQRINGLVDGISGNWYCKFDFT	180
PGSC0003DMG400004020	FTPAVERGLPVTVRISRMTLQQILARAVGDTIMNESNVDFEDDGEKVSVVLENGQRFIT	240
KJ-ZEP	FTPAVERGLPVTVRISRMTLQQILARAVGDTIMNESNVDFEDDGEKVSVVLENGQRFIT	240
PGSC0003DMG400004020	GDLLVGADGIRSKVRINLFGPSEVYSGYTCYTG IADFPVAD IDTVGYRVLGHKQYFVS	300
KJ-ZEP	GDLLVGADGIRSKVRINLFGPSEVYSGYTCYTG IADFPVAD IDTVGYRVLGHKQYFVS	300
PGSC0003DMG400004020	SDVGGGKMQWYAFVNEPAGGVDAPNGKKEKLLKIFGGWCDNVIDLVADEDAILRRDIY	360
KJ-ZEP	SDVGGGKMQWYAFVNEPAGGVDAPNGKKEKLLKIFGGWCDNVIDLVADEDAILRRDIY	360
PGSC0003DMG400004020	DRPPTFSWGRGHVTLGGDSVHAMQPNLQGGGCHAIEDSYQALALELDKACRSFAESGSPVD	420
KJ-ZEP	DRPPTFSWGRGHVTLGGDSVHAMQPNLQGGGCHAIEDSYQALALELDKACRSFAESGSPVD	420
PGSC0003DMG400004020	IISLRSYESARKLRVGVIHGLARMAAIMASTYKAYLVGLGFLSFLTQYRIPHPGRVGG	480
KJ-ZEP	IISLRSYESARKLRVGVIHGLARMAAIMASTYKAYLVGLGFLSFLTQYRIPHPGRVGG	480
PGSC0003DMG400004020	RVFIDLGHPLMLSVVLGGNGDKLEGR IKHCRLESEKANDQLRKFEDDALERATDAEWLL	540
KJ-ZEP	RVFIDLGHPLMLSVVLGGNGDKLEGR IKHCRLESEKANDQLRKFEDDALERATDAEWLL	540
PGSC0003DMG400004020	LPAGNGTSGLEAIVLSRDEDPCTIGSVSHNIPGKSVVLPQVSEMHARISCKDGAFF	600
KJ-ZEP	LPAGNGTSGLEAIVLSRDEDPCTIGSVSHNIPGKSVVLPQVSEMHARISCKDGAFF	600
PGSC0003DMG400004020	VTDLQSEHGTVWTDNEGRRYRTPNFPTFRFHPSDVIEFGSDKVAFRVKAMKFPKPTTERK	660
KJ-ZEP	VTDLQSEHGTVWTDNEGRRYRTPNFPTFRFHPSDVIEFGSDKVAFRVKAMKFPKPTTERK	660
PGSC0003DMG400004020	EEREAVGAA	669
KJ-ZEP	EEREAVGAA	669

Fig. 9: Pairwise alignment of the amino acid sequence of protein deduced from KJ-ZEP gene cloned from potato with that of available in Potato Genome Sequence Database (PGSC0003DMG400004020).

increasing the targeted carotenoid such as beta-carotene, violaxanthin, zeaxanthin, neoxanthin, lutein *etc.* in potato. Potato being third most important food crop after rice and wheat will have higher impacts of nutritional improved cultivar consumptions on consumers' health.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

## LITERATURE CITED

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# GENOTYPE AND ABSCISIC ACID INTERACTION ON TUBER DORMANCY AND SPROUTING DURING AMBIENT STORAGE IN POTATO

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**ABSTRACT:** Genotype and abscisic acid (ABA) interaction on tuber dormancy and sprouting were studied in the tubers of eleven potato genotypes during ambient storage. Tubers were harvested and hardened for ten days in the fields. Tuber weights were measured. Tubers were then stored after three treatments: 1) untreated tubers as such; 2) tubers treated with ABA solution, 3) tubers treated with water. During storage, dormancy length was measured. The longest sprout length and sprout numbers were measured at 30, 37, 42, 45, 50 and 60 d after storage. Tuber rottage was measured at 72 d after storage. Results found tubers of Lady Rosetta and Kufri Sindhuri having low tuber weights and longer dormancy duration while lesser sprouting and rottage compared to other genotypes. ABA treatment increased dormancy duration in Lady Rosetta, Kufri Neelkanth, Kufri Frysona, and Kufri Sindhuri. ABA treatment reduced sprouting in Kufri Neelkanth, Kufri Frysona, Kufri Chipsona 1, Kufri Ganga, Lady Rosetta, MP/06 39 and MS/8 1148. ABA treatment decreased rottage in Kufri Pukhraj, Kufri FryoM, Kufri Frysona, MS/8 1148, Kufri Neelkanth, Kufri Chipsona 1 and Kufri Sindhuri. Results indicated that tubers of Lady Rosetta and Kufri Sindhuri could be storable at ambient temperature compared to other genotypes. ABA treatment of tubers may improve tuber storage under ambient conditions in potato.

**KEYWORDS:** Abscisic acid, dormancy, sprouting, *Solanum tuberosum*

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is a non-grain food crop. A major problem in potato management is potato storage after harvest. After harvest, tubers are prone to post harvest damage as these are semi-perishable. In efforts to reduce such damage, tubers are stored in cold stores (Olsen *et al.*, 2009; Campbell *et al.*, 2014; Gupta *et al.*, 2015). In such commercial storages, chemicals like chlorpropham (CIPC) are used to suppress post-harvest sprouting. These efforts increase shelf life of tubers but increase the cost for farmers and raise societal and health concerns regarding the use of chemicals on eatables (Paul *et al.*, 2016). Extension of tuber dormancy in potato cultivars may help to improve shelf-life. Potato tuber dormancy is

an inherent trait (Olsen, 2009). To improve storage of potato tubers, longer dormancy can be built in potato cultivars by breeding programs. Few studies have shown that tuber weight can be the best variable related to dormancy length (Claassens and Vreugdenhil, 2000).

Potato tubers undergo three processes during storage, endodormancy, paradormancy and tuber aging. Endodormancy is due to endogenous factors, in which tubers do not sprout even under favorable conditions. Paradormancy is also known as apical dominance in which one leading sprout i.e., apical sprout grows and suppresses lateral sprouts to develop and grow. Tuber aging comes later in which extent of sprouting increases with multi-sprouts, and branches

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(Coleman, 2000; Sonnewald and Sonnewald, 2014).

Like seed dormancy, tuber dormancy related to ABA content (Destefano-Beltran *et al.*, 2006a; b; Campbell *et al.*, 2014; Muthoni *et al.*, 2014; Sonnewald and Sonnewald, 2014). Declined level of ABA during storage has been related to loss of dormancy and start of tuber sprouting (Campbell *et al.*, 2014; Muthoni *et al.*, 2014). Nitric oxide treatment of dormant tubers released tuber dormancy and induced sprouting while decreased ABA metabolism and signaling (Wang *et al.*, 2020). Therefore, ABA is having role in the maintenance of tuber dormancy during storage. Thus, applying ABA to potato tubers may increase dormancy thus could help in improving shelf life of potato tubers, however, is not well studied.

In the present study, the effect of genotype and ABA interaction has been studied during tuber storage in eleven potato genotypes where tubers were stored under ambient conditions. Four genotypes, Lady Rosetta, Kufri FryoM, Kufri Chipsona-1, Kufri Frysona were the known processing varieties. Lady Rosetta is exotic and red colored variety. Kufri Pukhraj and Kufri Ganga were the table purpose varieties for cultivation. Kufri Neelkanth and Kufri Sindhuri were the varieties developed for purple/red skinned tubers. MS/7 645, MP/06 39 and MS/8 1148 were the genotypes included in the study.

## MATERIALS AND METHODS

### Plant material

Tubers of potato (*Solanum tuberosum* L.) genotypes (Lady Rosetta, Kufri Pukhraj, Kufri FryoM, Kufri Neelkanth, MS/7 645, Kufri Ganga, Kufri Chipsona-1, Kufri Frysona, MP/06 39, MS/8 1148 and Kufri Sindhuri) were sown in the experimental farm of the Department of Vegetable Science, Punjab

Agricultural University, Ludhiana. Tubers were sown on ridges (20 cm tuber to tuber spacing and 60 cm row to row spacing) in plot (4 × 2.4 m<sup>2</sup>). Tubers were haulm killed at 90 days after sowing and harvested at 120 days after sowing. Harvested tubers were hardened under field conditions for 10 days then stored. Temperature and humidity measurements during crop growth were obtained from the Department of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana.

For storage, tubers were packed in jute bags and kept in the room. The temperature and humidity of the room during tuber storage were recorded on a daily basis. Tubers stored as such without any treatment were marked as untreated tubers. Tubers sprayed with 10 µM ABA solution were marked as ABA-treated while tubers sprayed with water were marked as water-treated (water control). Spraying was done six times at the interval of 15 minutes, tubers were then dried and stored.

### Measurement of tuber dormancy

Dormancy was considered the number of days after storage when 80% of tubers gained longest sprout length more than 2 mm.

### Measurement of tuber sprouting

Longest sprout length (LSL) and sprout numbers per tuber were measured at 30, 37, 42, 45, 50 and 60 days after storage (where n=30 tubers).

### Measurement of tuber weight

Tuber weight (n=15 tubers) were measured at the time of storage.

### Measurement of tuber rottage

The number of tubers rotted out of thirty tubers were measured at 72 days after storage.

## Statistical analysis

Data of sprouting were subjected to analysis of variance (ANOVA) followed by estimated marginal means (emmeans) measurements and then emmeans were separated by Fisher's L. S. D. at  $p \leq 0.05$  using R-studio. Box plot for tuber weight was generated using R-studio.

## RESULTS AND DISCUSSION

### Temperature and humidity variations during crop growth and during tuber storage

During crop growth, temperature decreased from about 24°C at the time of sowing to 12°C around the time of haulm killing then raised to about 15°C around the time of harvest then raised to about 20°C till the time of storage (Fig. 1a). Relative humidity varied from 60 to 95% during crop growth. During storage, temperature and humidity varied from 23-36°C and 40-70% respectively (Fig. 1b).

### Tuber dormancy

Three genotypes Kufri Sindhuri, Lady Rosetta and MS/7 645 showed longer dormancy length (Table 1) compared to others. Longer dormancy period for Kufri Sindhuri (>75 days) compared to Kufri Pukhraj, Kufri Chipsona 1 and Kufri Frysona (>45 days) has been reported (Gupta *et al.*, 2015) during ambient storage. ABA treatment increased dormancy length in four genotypes Lady Rosetta, Kufri Neelkanth, Kufri Frysona and Kufri Sindhuri. ABA is reported to be positive for tuber dormancy maintenance (Biemelt *et al.*, 2000; Destefano-Beltran *et al.*, 2006a; b).

### Tuber sprouting and tuber rottage

ANOVA results (Table 2) showed significant interactions among genotype (G), treatment (T) and storage duration (D) on longest sprout length (LSL) and sprout numbers.

**Table 1: Effect of genotype and ABA on dormancy length in eleven potato genotypes**

Genotype	Dormancy length after storage (days)		
	Untreated tubers	Water treated tubers	ABA treated tubers
Lady Rosetta	60	60	70
Kufri Pukhraj	40	40	40
Kufri FryoM	30	30	30
Kufri Neelkanth	35	35	42
MS/7 645	60	60	60
Kufri Ganga	50	50	50
Kufri Chipsona1	40	42	42
Kufri Frysona	35	35	43
MP/06 39	50	50	50
MS/8 1148	40	45	45
Kufri Sindhuri	60	60	>70

†Values are the number of days after storage when 80% of tubers (i.e., 24 tubers out of 30 tubers) showed longest sprout length  $\geq 2$  mm.

††Tubers were harvested after 30 days of haulm cutting and hardened in fields for 10 days, then treated with water (as control) and 10  $\mu$ M ABA solution then dried and stored. Untreated tubers stored directly without any treatment.

**Table 2: ANOVA result showing effect of genotype (G), treatment (T) and duration of storage (D) and their interaction on longest sprout length and sprout number per tuber**

Effect	Df	MS	
		longest sprout length tuber <sup>-1</sup>	sprout number tuber <sup>-1</sup>
G	10	1613***	64.62***
T	2	19	46.78***
D	5	3578***	263.8***
G: T	20	124***	7.61***
G: D	50	92***	3.71***
T: D	10	53***	6.08***
G: T: D	100	15***	1.18***
Residuals	5742		

†Significant effects at  $p \leq 0.001$ , 0.01 and 0.05 shown as \*\*\*, \*\* and \* respectively.

LSL (Table 3) remained low in Kufri Sindhuri, Lady Rosetta and MS/7 645 compared to other genotypes during storage. ABA treatment decreased LSL in Kufri Neelkanth at 42, 45, and 50d compared to

**Table 3: Effect of ABA treatment on longest sprout length in tubers of eleven potato genotypes at different days (d) after storage at ambient temperature**

Duration	Treatment	Genotypes										
		Lady Rosetta	Kufri Pukhraj	Kufri FryoM	Kufri Neelkanth	MS/7 645	Kufri Ganga	Kufri Chipsona1	Kufri Frysona	MP/06 39	MS/8 1148	Kufri Sindhuri
30 d	UT	0	0.2	2.24	1.24	0.04	0.1	0.64	0.7	0.3	0	0
	WT	0	0.15	2.05	1.5	0.03	0.3	0.65	2.22	0.35	0	0
	ABA	0	0.4	2.37	0.33	0.03	0	0.72	0.65	0.8	0.12	0
37 d	UT	0.12	1.23	3.15	2.55	0.29	0.42	1.14	2.07	0.72	0.47	0
	WT	0.13	2.07	3.52	2.57	0.28	0.93	0.98	4.23	1.1	0	0
	ABA	0.03	1.65	3.97	1.4	0.23	0.17	1.4	1.52	1.38	0.53	0
42 d	UT	0.4	2.24	3.87	3.14	0.57	1.27	2.12	3.25	1.29	1.25	0.09
	WT	0.45	3.17	3.77	4.53	0.57	1.15	1.42	6.43	1.4	1.55	0
	ABA	0.23	3.62	7.1	2.27	0.38	0.33	1.88	3.17	1.9	1.38	0
45 d	UT	1.02	4.88	5.77	4.38	1.17	2.23	4.88	5.65	1.67	3.25	0.27
	WT	0.95	3.82	4.33	5.52	1.17	1.65	3.63	7.5	2.27	3.23	0.63
	ABA	0.88	6.37	9.08	3.63	1.27	1.58	3.67	5.77	3.3	3.1	0.03
50 d	UT	1.7	5.62	6.2	5.53	1.7	3.08	5.75	6.63	2.47	4.03	0.37
	WT	1.73	5.6	5.25	6.62	1.7	2.72	5.17	8.7	2.88	3.85	0.73
	ABA	1.75	8.2	10.15	4.87	2.18	2.47	4.63	6.38	4.03	3.57	0.13
60 d	UT	2.47	7.8	8.27	9.7	2.27	5.58	7.93	10.38	5.03	13	1.22
	WT	4.12	6.07	5.1	8	2.27	3.2	6.32	8.1	3.13	6.57	1.83
	ABA	2.47	9.87	11.13	5.57	2.23	3.53	6.37	8.17	5.2	6.93	0.22
L. S. D. at $p \leq 0.05$						1.51						

†Values are the estimated marginal means separated by Fisher's L. S. D. at  $p \leq 0.05$ .

††Tubers were harvested after 30 days of haulm cutting and hardened in fields for 10 days, then treated with water (WT as control) and 10  $\mu$ M ABA solution then dried and stored. Untreated tubers (UT) stored directly without any treatment.

water control and at 60d compared to both water and untreated controls. ABA decreased LSL in Kufri Frysona at 30, 37, 42, 45 and 50 d compared to water control. ABA decreased LSL in Kufri Ganga, Kufri Chipsona 1 and MS/8 1148 at 60 d compared to untreated control. ABA decreased LSL in Lady Rosetta and Kufri Sindhuri at 60d compared to water control. However, ABA treatment increased LSL at 45d compared to water control and at 50 and 60d compared to both water and untreated controls in Kufri Pukhraj and at 42, 45, 50 and 60 d compared to both water and untreated controls in Kufri FryoM. In MP/06 39 also, ABA treatment increased LSL at 45 and 50d compared to untreated

control and at 60d compared to water control.

Sprout numbers (Table 4) were seen low in Lady Rosetta and Kufri Sindhuri compared to other genotypes during storage. ABA treatment reduced sprout numbers in Lady Rosetta at 42d compared to both water and untreated controls. ABA treatment decreased sprout numbers in Kufri Ganga at 37 and 42 d and in Kufri Chipsona 1 at 45, 50 and 60d and in MS/8 1148 at 37, 42, 45, 50 and 60d and in Kufri Frysona and MP/06 39 at 60d compared to untreated control. However, ABA treatment increased sprout numbers at 45, 50 and 60d in Kufri Pukhraj and at 45 and 50d in Kufri FryoM compared to both

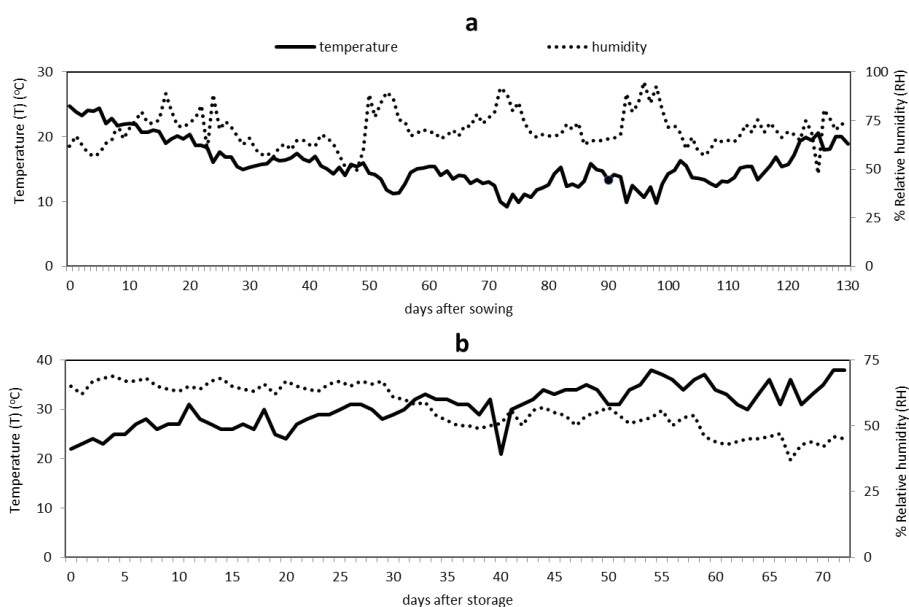


Fig. 1. Temperature and humidity during crop growth at different days after sowing (a) and during tuber storage at different days after storage (b)

Table 4: Effect of ABA treatment on sprout numbers in tubers of eleven potato genotypes at different days (d) after storage at ambient temperature

Duration	Treatment	Genotypes										
		Lady Rosetta	Kufri Pukhraj	Kufri FryoM	Kufri Neelkanth	MS/7 645	Kufri Ganga	Kufri Chipsona1	Kufri Frysona	MP/06 39	MS/8 1148	Kufri Sindhuri
30 d	UT	0	0	0.47	0.2	0.07	0.03	0.23	0.43	0.13	0	0
	WT	0	0.07	0.6	0.4	0.07	0.1	0.17	0.73	0.17	0	0
	ABA	0	0.23	0.67	0.1	0.03	0	0.28	0.47	0.3	0.1	0
37 d	UT	0.17	0.77	0.63	0.7	0.47	0.43	0.63	0.83	0.4	0.53	0
	WT	0.17	0.57	0.69	0.63	0.47	0.2	0.4	0.83	0.23	0	0
	ABA	0.07	0.8	0.8	0.77	0.37	0.13	0.43	0.77	0.4	0.23	0
42 d	UT	0.53	0.9	0.73	0.73	0.9	0.83	0.83	0.93	0.47	0.77	0.13
	WT	0.57	0.73	0.77	0.93	0.9	0.23	0.43	0.9	0.23	0.37	0
	ABA	0.23	1	1	0.83	0.93	0.27	0.57	0.97	0.43	0.3	0
45 d	UT	0.8	1.77	1.17	1.37	1.33	0.97	2.27	1.97	0.9	1.43	0.2
	WT	1	0.77	0.8	1.17	1.33	0.57	1.13	1.63	0.37	0.93	0.27
	ABA	0.9	2.27	1.53	1.2	1.3	1.17	1.47	2.07	0.9	1.07	0
50 d	UT	1.07	1.83	1.27	1.47	1.4	1.13	2.3	1.97	1.33	1.5	0.3
	WT	1.23	1.57	0.87	1.17	1.4	1.3	1.15	1.6	0.67	1.07	0.3
	ABA	1.03	2.57	1.53	1.43	1.5	1.43	1.5	2.07	1.13	1.17	0.27
60 d	UT	1.1	2.13	1.77	2	1.53	1.43	2.73	2.63	1.8	2.53	0.4
	WT	1.13	1.43	0.93	1.93	1.47	1.4	1.2	2.1	0.73	1.07	0.47
	ABA	1.33	2.93	1.63	1.83	1.52	1.37	1.7	2	1.2	1.27	0.47
L. S. D. at $p \leq 0.05$							0.3					

†Values are the estimated marginal means separated by Fisher's L. S. D. at  $p \leq 0.05$ .

††Tubers were harvested after 30 days of haulm cutting and hardened in fields for 10 days, then treated with water (as control) and 10  $\mu$ M ABA solution then dried and stored. Untreated tubers stored directly without any treatment.

water and untreated controls. Moreover, ABA treatment increased sprout numbers compared to water control but not compared to untreated control in Kufri Chipsona 1 and MP/06 39 at 45, 50 and 60d and in Kufri Frysona at 45 and 50d. In fact, water treatment reduced sprout numbers while ABA treatment increased sprout numbers in these samples.

Tuber rottage (Table 5) was not observed in untreated tubers of Lady Rosetta, MS/7 645 and Kufri Sindhuri. ABA treatment decreased rottage in Kufri Pukhraj, Kufri FryoM, Kufri Frysona, MS/8 1148 compared to both untreated and water-treated controls. ABA treatment decreased rottage in Kufri Neelkanth, Kufri Chipsona 1 and Kufri Sindhuri compared to water control. Compared to untreated tubers, rottage was relatively high in water treated tubers in many genotypes (Kufri Pukhraj, Kufri Neelkanth, Kufri Ganga, Kufri Chipsona 1, Kufri Frysona, MS/8 1148 and Kufri Sindhuri).

ABA levels were reported to be highest in the dormant tubers at the time of harvest and levels decreased during storage (Suttle *et al.*, 2012). However, treatment of dormant tubers

with exogenous ABA showed no appreciable effect on dormancy duration (Suttle *et al.*, 2012). El-Antably *et al.* (1967) indicated that ABA inhibited sprout growth when applied to the whole tubers, however, was much less effective when applied to isolated tuber plugs. Wang *et al* (2020) reported that spraying dormant tubers with 100 µM solution of ABA decreased nitric oxide (NO) content at 3 and 6 hours and reduced tuber sprouting at 20, 30, 40 and 60 days after treatment compared to water control. Present study indicated that ABA can be considered for enhancing dormancy duration in potato. In the present study, 10 µM solution of ABA was used and treatment was given once. Recently, post-harvest treatment of zucchini fruits with 500 µM solution of ABA alleviated chilling injury through activation of antioxidant defense (Castro-Cegrí *et al* 2023). In future, concentration of ABA may be increased. Secondly, water treatment relatively affected tuber quality compared to untreated samples, thus ABA may be given in gaseous form (fumigation, aerosol or dust).

In Kufri Pukhraj and Kufri FryoM, ABA treatment increased sprouting but reduced rottage. Tuber aging might be prevented by ABA. In *Phaseolus coccineus*, age-related effects of ABA were observed where in young stem, ABA promoted apical dominance but in aged stem, ABA decreased apical dominance and promoted lateral branching (Hartung and Funfer, 1981). Subapical necrosis develops on sprouts in aged tubers which are prevented by low temperature treatment or by calcium application (Coleman, 2000). Low temperature storage increases endodormancy but decreases paradormancy in potato tubers (Eshel and Teper-Bamnlker, 2012; Teper-Bamnlker *et al.*, 2012). Though ABA content decreased during tuber storage but decline in ABA was not prerequisite for dormancy exit and the onset of tuber sprouting (Biemelt

**Table 5: Number of rotted tubers out of thirty tubers in potato genotypes at 72 days after storage under ambient conditions**

Genotype	Number of rotted tubers out of thirty		
	Untreated tubers	Water treated tubers	ABA treated tubers
Lady Rosetta	0	0	0
Kufri Pukhraj	5	13	0
Kufri FryoM	10	10	3
Kufri Neelkanth	6	10	8
MS/7 645	0	0	0
Kufri Ganga	4	8	8
Kufri Chipsona1	4	8	4
Kufri Frysona	7	13	5
MP/06 39	7	6	7
MS/8 1148	5	7	4
Kufri Sindhuri	0	1	0

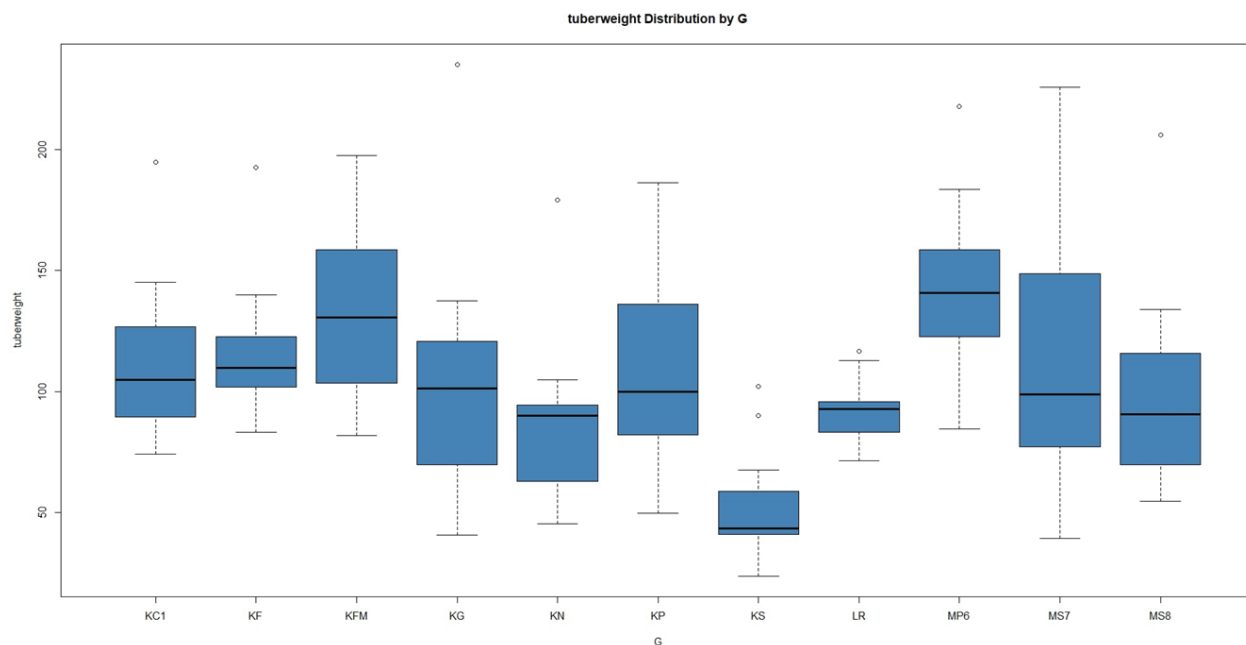


Fig. 2. Box plot showing tuber weight distribution by genotypes. Tuber weight ( $\text{g tuber}^{-1}$ ) listed on y-axis while genotypes listed on x-axis. KC1, Kufri Chipsoan 1; KF, Kufri Frysona; KFM, Kufri FryoM; KG, Kufri Ganga; KN, Kufri Neelkanth; KP, Kufri Pukhraj; KS, Kufri Sindhuri; LR, Lady Rosetta; MP6, MP/06 39; MS7; MS/7 645; MS8, MS/8 114

*et al.*, 2000; Suttle *et al.*, 2012). In addition, as sprouting approached, an unexpected increase of ABA in eyes and sub-eye regions of tubers was also reported (Soruce *et al.*, 1996). Thus, ABA may be promoting dormancy and reducing sprouting in dormant tubers. However, in sprouted tubers, ABA may enhance sprouting to prevent tuber aging.

### Tuber weight

Box plot was created to visualize the distribution of tuber weight for each genotype (Fig. 2). Mean tuber weights were seen low in Lady Rosetta, Kufri Sindhuri and Kufri Neelkanth however were high in other genotypes including MS/7 645. Standard deviations (i.e., length of box plot) can be seen very high in other genotypes compared to Lady Rosetta and Kufri Sindhuri.

Ittersum and Struik (1992) found correlation coefficient = -0.82 between tuber weights and duration of tuber dormancy. The physiological age of tubers varied with the

size of tubers. Tubers with diameter smaller than 35 mm had longer dormancy duration than tubers larger than 35mm (Müller *et al* 2010). Large tubers (50-60 g) exhibited faster dormancy release than smaller (10-20 g) and medium sized (30-40 g) tubers (Park *et al* 2023). Tuber weights were low in Lady Rosetta and Kufri Sindhuri however in MS/7 645, tuber weights were high compared to Lady Rosetta and Kufri Sindhuri. Uniformity of tuber size may also be an important trait as observed in Lady Rosetta and Kufri Sindhuri compared to other genotypes.

### CONCLUSION

This study concluded that Lady Rosetta and Kufri Sindhuri were storable genotypes compared to other genotypes under ambient conditions. Tuber size may be an important trait related to tuber dormancy. Uniformity in tuber size may also be an important trait for tuber dormancy. ABA treatment can be used for enhancing dormancy and reducing

sprouting and tuber rottage during ambient storage.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

### ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

### DATA AVAILABILITY

All data generated or analysed during this study are included in this article. Requests for material should be made to the corresponding author.

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# EVALUATION OF POTATO *IN VITRO* PLANTS WITH DIFFERENT INTER AND INTRA-ROW SPACING IN SOILLESS SYSTEM UNDER INSECT PROOF NET HOUSE

Sugani Devi\*, Khushdil Bharti, Ratna Preeti Kaur, Brajesh Nare and Sukhwinder Singh

**ABSTRACT:** Availability of disease-free quality seed material is the urgent need for improving potato productivity. *In vitro* plants of potato are the pure form of seed and are free from disease pest contamination. The present study was undertaken under an insect-proof net house to evaluate the performance of microplants of two potato varieties (*Kufri Pukhraj* and *Kufri Himalini*) at three different planting densities. The results of the experiment reveal that for getting the maximum number and yield of mini-tubers from *in vitro* plantlets under soilless culture in insect-proof net house, we should increase planting density to 66 plants per m<sup>2</sup> (spacing of 15X10 cm). In a mini-tuber production scheme, it is always important to get yield in terms of numbers in relation to the size. The multiplication rate in potato seed production is low (4-5 times), hence it is always desirable to increase the number of tubers produced at harvest even if some compromise has to be done with the size of tubers. Since both the number and weight of minitubers obtained was higher at closer spacing, it is better to reduce the spacing to accommodate more plants resulting in the production of more number of tubers in the same area.

**KEYWORDS:** *Solanum tuberosum*, microplants, aeroponic tubers, plant geometry, early generation seed

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is third most economic non cereal crop after rice and wheat in India. Potato has been identified as a future crop for meeting food security issues (Singh and Rana, 2013). It contributes as important source of income across the world's farming community. Potato provides food and nutritional security and also helps to eliminate poverty in developed and developing countries. India has emerged as the second largest producer of potato in the world after China with contribution of 13.54% in production and 11.24% in area in world potato cultivation (NHB, 2019 and FAOSTAT, 2017). Potato can be grown from seed tuber size ranging from 30 to 55 mm diameter and require 2-3 t/ha average seed rate. In Asia, India is only a country which have well predictable seed production program and ICAR-CPRI singly produces breeder seed

about 3,000 metric tonnes annually. In the last 65 years, country has made marvellous progress in the production of potato due to release of number of high yielding varieties which are resistant to different disease or pest with advanced technological interference, appropriate for different agro-climatic condition of the country. Seed Plot Technique development contributes massively to the production of quality seed potato on large scale. Keeping in view the deteriorating potato seed health under field conditions, quality seed production can be increased by adopting measures to check seed stock degeneration. Struik and Wiersema (1999) directly correlated high potato yields with use of high quality, certified seed tubers.

Hi tech seed production systems advanced in recent years satisfactorily counter the issues of low rate of seed multiplication under conventional seed production system

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(Badoni and Chauhan, 2010) and increasing demand of quality seed material. Hi tech seed system includes different rapid multiplication techniques like tissue culture, net house multiplication and aeroponics. The disease-free tested potato microplants are multiplied in tissue culture followed by its further multiplication in net house or aeroponics to produce G0 minitubers. Now the problem with aeroponics is that it is very expensive and not affordable to small and medium farmers and need technical knowledge for crop management. In vector proof net house in spite, of a longer cropping season in northern India lasting from October to April, only one potato seed crop is being grown in one crop season in soil as there is risk of soil borne diseases in successive dual crop. Technologies and strategies to increase productivity at the earliest seed stages can have profound effect on overall seed production (Kaur *et al.*, 2019). There are different types of alternate media that plants can grow as well in it. For potato production, cocopeat as soilless media is most popular which is made from cocopeat fiber powder. Having different properties of cocopeat includes as easy absorbing and saving water and also have pores that facilitate the exchange of air resulting provide favourable condition for root development of plant. So, we can use soilless media like cocopeat which can be successfully sterilized between two crop cycles and the precious net house area can be proficiently utilized by taking multiple crops. The technical information is still lacking in potato for soilless minitubers production like crop geometry, nutrients, crop duration; crop management practices *etc.* which can be further improve the productivity of early generation seed material. For any potato seed program, obtaining many medium sized mini-tubers is more important than producing few large mini-tubers (Love *et al.*, 2003). Therefore, many

studies have been conducted to establish the optimal combination of seed size and planting distance for a certain environment (Entz and La Croix, 1984; Negi *et al.*, 1995; Bussan *et al.*, 2007). They concluded that yield increased with choosing lesser plant spacing. Keeping in view these facts the study was conducted to find out the best planting geometry under cocopeat medium for maximization of multiplication rate of disease free minitubers from *in vitro* plants.

## MATERIALS AND METHODS

The study was conducted at CPRS, Jalandhar in insect-free net house to find out the best planting geometry for tissue cultured potato plants under cocopeat medium. Hardened microplants of two varieties (K. Himalini and K. Pukhraj) were shifted in soilless beds on 5 November 2020 in insect proof net house with three plant densities (PDs) replicated three times.

### *In vitro* propagation of plantlets

Only virus free plantlets propagated through micropropagation were used. *In vitro* plants were propagated routinely at tissue culture laboratory at CPRS, Jalandhar. Single node cuttings were sectioned and placed in test tubes (1 cutting per tube) on fresh MS medium (Murashige and Skoog, 1962). Sub culturing of microplants was performed once every 3-4 weeks. The temperature in growth room was 20-22°C; photoperiod was 16/8 h day and night respectively.

### Planting of *in vitro* plantlets and crop husbandry practices in soilless media under insect proof net house

The longer adventitious roots of microplants were trimmed off slightly and then planted in pro trays in coco-peat media and hardened for about 20 days. Hardened microplants of two varieties (K. Himalini and K. Pukhraj) were shifted in

soilless beds at four PDs- 22 plants  $m^{-2}$  ( $D_1$ ), 44 plants  $m^{-2}$  ( $D_2$ ) and 66 plants  $m^{-2}$  ( $D_3$ ) respectively. Before planting cocopeat media was sterilized with Nano silver hydrogen peroxide 3 percent solution by drenching followed by thoroughly washing with clean water. Growing containers (troughs) of HDPE sheet of 700 micron thickness was placed on a raised bench and used for planting of *in vitro* plantlets. For nutrient management in cocopeat soilless culture, ICAR-CPRI liquid formulation containing macro and micro essential nutrient elements was given after dilution with water to maintain EC range between 1.5-2.0 and pH range of 6.0 to 6.5. All cultural practices recommended for the region for net house cultivation were followed with haulm cutting at approximately 90 days in all the treatments.

### Experimental design and statistical analysis

Experiment was carried out with factorial RBD with two factors; varieties (K. Pukhraj & K. Himalini) and planting densities ( $D_1$ ,  $D_2$  and  $D_3$ ) with three replications. Observations were recorded on various plant growth and yield parameters. Survival percentage was recorded at 30 days after shifting in net house (DAP). Growth observations for five plants in a single replication were recorded at 30 and 60 days to evaluate the growth pattern of two varieties in different crop geometries. It was further averaged to derive the respective values for each replication. Harvested tubers were classified into two categories undersize (below 3 g) and seed size (3- 30 g). Category of oversize tubers (>30 gm) was not considered as none of the tubers were found above 30 g due to closer row to row planting of 30 cm. Tuber number and yield of under size and seed size tubers were depicted as NUT (Number of undersize tubers/  $m^2$ ) and WUT (weight of undersize tubers/  $m^2$ ) and NST (Number

of seed size tubers/  $m^2$ ) and WST (weight of seed size tubers/  $m^2$ ), respectively. Similarly, total number and weight of the tubers were depicted as TN (total tubers/  $m^2$ ) and TW (total weight of tubers/  $m^2$ ). The average was analyzed statistically by applying the technique of analysis of variance (ANOVA). Mean values were calculated and separated using F-test at 5% level of significance.

## RESULTS AND DISCUSSION

### Total number of tubers per $m^2$ (TN)

Tuber number is an important character which determines the multiplication rate in potato and is most crucial for seed potato production. Planting density of *in vitro* plants and cultivar had significant effect on number of minitubers per  $m^2$  and tuber yield (Table 2). This finding confirms results obtained by Kumar *et al.*, 2012 and Kaur *et al.*, 2019. No significant interaction effect was found between varieties and PDs. The variety Kufri Pukhraj (446.28) significantly outyielded variety K. Himalini (259.94) for total number of tubers produced per  $m^2$ . The characters showed significant differences among different PDs. As expected earlier significantly maximum tuber number per  $m^2$  was obtained with higher plant density  $D_3$  (482.46) followed by  $D_2$  (354.42) and minimum with  $D_1$  (222.46), the lowest plant density. Increase in number at higher plant density might be due to the corresponding higher number of stems available per unit area on account of more number of plantlets planted. More number of tubers per unit area with increasing plant population has been reported to occur on account of more number of stems per unit area in conventionally grown potato (Kumar *et al.*, 2011; Kushwah and Singh, 2008; Malik *et al.*, 2000 and Zamil *et al.*, 2010) as well as in the mini-tubers raised from *in vitro* plantlets (Kumar *et al.*, 2012).

### Yield per square meter (TW)

The yield is indicative of the total weight of tubers obtained per metre square for different planting density treatments. Significant differences for yield over variety and density were obtained (Table 2). The significantly higher yield was obtained for the variety Kufri Pukhraj as compared to variety Kufri Himalini. Among the PD treatments included in the study D2 and D1 showed at par yields, which were however significantly lower than the D3 treatment means. Comparatively higher proportions of large sized mini-tubers obtained at low plant density and vice-versa can be attributed to the increasing competition between plants for space and nutrients coupled with higher number of mini-tubers obtained per unit area at high plant density. Whereas, per plant tuber yield is significantly higher at lower plant density and vice versa.

### Seed size tuber number per m<sup>2</sup> (3-30 g)

Seed size mini-tubers indicate the economically most important class of tubers with respect to seed multiplication and vigour. The character number of seed size

tubers depicted highly significant variations for variety and planting densities (Figure 1). Significantly higher tuber number was obtained in variety K. Pukhraj (347.85) than K. Himalini (224.01) on per m<sup>2</sup> basis. Higher planting density resulted in higher seed size minitubers per m<sup>2</sup>. Among the PDs treatments, maximum number of seed size tubers was seen in D3 treatment which was significantly higher than the other PDs considered in the experiment, showing a 101.18% increase over D1. However, when we observed the data regarding number of seed size tubers per plant results are just opposite to per m<sup>2</sup> data. Seed size tuber number per plant was significantly higher in wider spacing D1 (8.61) which was followed by D2 (6.53) and D3 (5.78).

Increased or more number of tubers at high densities may be due to the fact that at low density plantings fewer sinks are produced per unit area and they increase with increasing planting density (Mangani *et al.*, 2015).

### Undersize tuber number per m<sup>2</sup>

Number of undersize tubers per m<sup>2</sup> was observed to be significantly influenced by

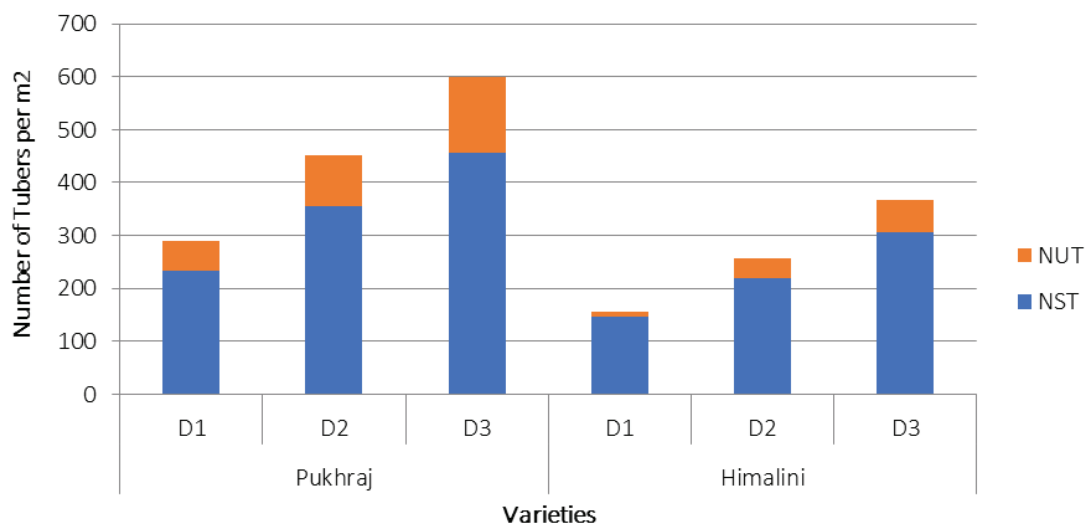


Fig. 1: Tubers number in different seed categories out of total tubers produced undersize (NUT) and seed size (NST) minitubers on per square meter basis for the two varieties Kufri Pukhraj and Kufri Himalini.

**Table 1. Effect of different plant spacing on growth parameters in soilless culture in potato**

Treatments/Observations	Plant Height (30 DAP)	Plant Height (cm) @ 60 DAP	No of leaves @ 30 DAP	No of leaves@ 60 DAP	SD (mm)
Varieties					
K. Pukhraj	13.81	38.22	12.78	13.29	3.84
K. Himalini	23.04	51.48	13.96	12.48	2.26
LSD (0.05)	2.315	3.86	0.978	NS	0.284
SEm±	0.725	1.210	0.306	0.318	0.089
Plant Density					
30X15(D1)	18.72	42.72	14.17	14.83	3.39
15X15 (D2)	19.22	45.28	13.17	11.72	2.89
15X10 (D3)	17.33	46.56	12.78	12.11	2.87
LSD (0.05)	NS	NS	NS	1.242	0.348
SEM	0.888	1.482	0.375	0.389	0.109

**Table 2. Effect of different plant spacing on yield parameters in soilless culture in potato**

Treatments/Observations	Number of seed size tubers per plant	tuber weight (g) per plant)	NST	NUT	TW
Varieties					
K. Pukhraj	8.51	79.74	347.85	98.44	3003.22
K. Himalini	5.43	76.35	224.01	35.93	2846.10
LSD (0.05)	0.608	NS	22.566	31.784	NS
SEm±	0.191	3.547	7.070	9.958	138.481
Spacing					
30X15(D1)	8.61	120.95	189.46	33.00	2660.79
15X15(D2)	6.53	61.67	287.17	67.25	2713.33
15X10(D3)	5.78	51.53	381.15	101.31	3401.20
LSD (0.05)	0.745	13.867	27.637	38.928	541.338
SEm±	0.233	4.344	8.659	12.196	169.604

variety and planting density. Significantly higher tuber number of this category was depicted in variety K. Pukhraj (98.44) than in K. Himalini (35.93) on per m<sup>2</sup> basis. Regarding impact of PDs, significantly highest number of undersize tubers were obtained in narrower spacing D3 (101.31) and the minimum as expected were for lower planting density D1 (33.0). It was therefore observed that higher number of undersize minitubers is formed at higher density, but their proportion out of total also increases simultaneously. The proportion of undersize tubers out of total

produce was higher in denser planting of 66 plants per m<sup>2</sup>D3 (20.99%) than lower plant density of 22 plants per m<sup>2</sup> (14.83%). Although, tubers of this category are not preferred as seed but are viable up to a certain lower limit below which the viability of minitubers may be lost due to shrinkage in storage and are further recycled/multiplied.

## Growth parameters

### Plant height

The variety Kufri Himalini showed higher plant height than Kufri Pukhraj (Table 1). The

maximum plant height was obtained in D3, though it was not significantly tall than all the other treatments included in the study which were at par. This gives an inference that the plants grew taller in response to higher density in face of competition for more light.

### *Number of leaves per plant*

The character showed (Table 1) significant variation for varieties at 30 DAP and for plant density at 60 DAP and not for their interactions. The significantly higher number of leaves per plant at 30 DAP were observed in the variety Kufri Himalini (13.96) than Kufri Pukhraj (12.78) because of more plant height. The plant density treatment D1 showed the maximum number of leaves (14.83) at 60 DAP followed by D3 and D2 which were at par statistically. The observation corresponds to more aerial space for growth of canopy in D1.

### *Stem diameter (SD)*

Stem diameter was measured at 30 DAP and was significantly affected by variety as well as by plant densities (Table 1). It was observed that stem diameter of plants of Kufri Pukhraj (3.84 mm) was significantly higher than Kufri Himalini (2.26 mm). Regarding stem diameter in different plant spacing, it was found that significantly highest diameter was observed in D1 (3.39 mm) plant spacing followed by D2 (2.89 mm) and D3 (2.87 mm) which are found at par to each other. This may be due to increased competition among the plants for space and other nutrients resulting in weak stems under narrow spacing treatment.

## CONCLUSION

The results of the experiment reveal that for getting maximum number and yield of mini-tubers from *in vitro* plantlets under soilless culture in insect proof net house, we should increase planting density to 66

plants per m<sup>2</sup> (spacing of 15X10 cm). In a mini-tuber production scheme, it is always important to get yield in terms of numbers in relation to the size (Park *et al.*, 2009). The multiplication rate in potato seed production is low (4-5 times), hence it is always desirable to increase the number of tubers produced at harvest even if some compromise has to be done with the size of tubers.

Multiplication rates achieved in the early seed multiplication generation has greater implication during subsequent seed multiplication generations. The multiplication of early generation seed up to 6-8 times before supply to farmers is generally practiced in India. Based on the results of the present experiment it can be postulated that a small intervention by increasing planting density under cocopeat medium in net house in early seed multiplication generations can be used favorably and economically to increase minituber production. Since both number and weight of minitubers obtained was higher at closer spacing, it is better to reduce the spacing to accommodate more number of plants resulting in production of more number of tubers in the same area.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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# PRODUCTION POTENTIAL OF AEROPONIC POTATO AS INFLUENCED BY DIFFERENT PLANTING AND IRRIGATION METHODS IN NORTH-CENTRAL INDIA

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**ABSTRACT:** Aeroponic is well established technology for more efficient and high quality seed potato production. An experiment was carried out at Central Potato Research Institute, Regional Station, Gwalior (MP) to find out the suitable method of planting and irrigation for further multiplication of aeroponic minitubers under net house conditions with six treatment combinations. Aeroponic minitubers of two popular potato variety of the region *viz* Kufri Lauvkar and Kufri Surya were planted with two types of seed beds *viz* flat bed and ridge and furrow with 30 cm × 10 cm spacing in which sprinkler and furrow irrigation method was applied. Among planting beds, significantly higher emergence% was recorded in ridge and furrow planting (87.22) over flatbed planting method (83.89). Weight of tubers was significantly higher in sprinkler irrigation (105.7 q/ha), ridge and furrow planting (107.8 q/ha) and variety Kufri Lauvkar (114.1q/ha) over furrow irrigation (98.3 q/ha), flat bed planting (96.2 q/ha) and variety Kufri Surya (89.8 q/ha) respectively. Significantly higher seed size (40-80g) tubers were recorded in sprinkler irrigation method (3% by number and 12.6% by weight) and variety Kufri Lauvkar (3.6% by number and 17.6% by weight) over furrow irrigation and Kufri Surya. Therefore, for higher production, minituber multiplication should be done in ridge and furrow method of planting with sprinkler irrigation under North-Central conditions of India.

**KEYWORDS:** aeroponic minitubers, planting methods, irrigation methods, variety, potato

## INTRODUCTION

Aeroponics is a modern, soilless technology and a viable alternative for the potato minituber production in which cultivar plays a significant role in the number of tubers formed (Rykaczewska, 2016; Bročić *et al.*, 2021). Aeroponic systems for prebasic potato seed production was established following increased demand for more efficient, high quality seed production methods (Ritter *et al.*, 2001, Nichols, 2005) and has a potential of revolutionizing potato seed production industry (Buckseth and Singh 2018) which has been well integrated in India for seed production (Sadawarti *et al.*, 2021). Net

houses are used in hi-tech seed production for cultivation of microplants as well as for recycling of small minitubers to get seed size or larger tubers for subsequent field multiplication and channeling into seed production generations (Kaur *et al.*, 2019). Tuber number and the size of the tubers is important for potato production. Seed size tubers are more prolific and economically more adaptive than underweight and overweight tubers. (Kaur *et al.*, 2019)

Potatoes are one of the most water-efficient crops, however, water management in potatoes is crucial as potato is one of the most water stress-sensitive crops due to its

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shallow rooting system and the sensitivity of the potato foliage characteristics. At present surface irrigation (furrow irrigation) in potato cultivation practice is less efficient compared to pressurized irrigation system. The water can be efficiently utilized by adopting the modern pressurized micro irrigation systems. Now a days, the sprinkler irrigation method is gaining popularity among the farmers which also used to maintain the healthy microclimate in the close growing crops along with irrigation (Chicham *et al.*, 2022). Sprinkler irrigation method distributes water to crops by spraying it over the crop area like a natural rainfall. It is estimated that the sprinkler irrigation method substantially reduces the use of water and the crop productivity also increases (Patel and Parmar, 2020).

Flat beds potato planting are more conducive for higher planting densities to reduce average tuber size (Mundy *et al.*, 1999). Flat beds offer benefits of capturing rainfall or irrigation water more efficiently (Robinson, 1999), and there are less fluctuations in temperature throughout the bed providing an insulating factor which promotes early emergence of crop (Mundy *et al.*, 1999) and also offer the scope for manipulating planting densities. Sharma *et al.* (2014) observed significantly higher number of minitubers for flatbed planting during standardization of plant densities and method (flat beds versus ridge and furrow) for microplant establishment under net house conditions of Northern hills. The classification of the tubers based on weight is of immense importance as the smaller tubers, tend to develop small and lesser number of sprouts, shoots, tubers, yield, prone to virus incidence and higher weight loss in storage as compared to larger sized tubers reportedly being less vigorous (Wurr *et al.*, 1992, Karafyllidis *et al.*, 1996). Simultaneously, very large size or oversize tubers are economically prohibitive as seed

and also more prone to diseases (Schotzko *et al.*, 1983; Rex and Mazza 1989). Keeping this in mind, a study was conducted (2018-19) to analyze the effect of irrigations methods and planting methods on production potential on potato minitubers under net house conditions of North-Central India.

## MATERIALS AND METHODS

An experiment was conducted in the net house of ICAR-Central Potato Research Institute, Regional Station, Gwalior (26° N and 78° E, altitude 207 m msl) with two early cultivar *viz* Kufri Lauvkar and Kufri Surya in 2018–19. Aeroponic minitubers were allowed to sprout under diffused light and planted in the 2<sup>nd</sup> week of November. Seed bed was prepared with layer of well decomposed farm yard manure and sand in the ratio of 1:1 on the top of the bed under insect-proof net house and two types of seed beds *viz* flat bed and ridge and furrow were used for trial. Well sprouted aeroponic minitubers were planted 30 cm × 10 cm in both the type of beds with 3 rows (2 m) which maintained the planting density of 333333 plants/ha. Both the beds were irrigated with sprinkler and flood furrow irrigation in ridge and furrow. The trial was planted in split plot design with three replications with irrigation method as main plot and bed method and varieties as sub plots. Trial was conducted as per standard seed plot techniques in which N:P:K was given in the ratio of 150:60:100. Full doses of P through single super phosphate, K through muriate of potash and half dose of N through ammonium sulphate were applied at the time of planting. Remaining half dose of N was applied through urea after 25-30 days of planting. Standard seed production practices were followed for the management of the crop. The emergence was recorded at 30 days after planting (DAP). The crop was terminated at 90 days by uprooting haulms and harvested after allowing skin curing for 15 days. Yield, total number of tubers, tuber grades, *viz.*

undersize <3g, 3-10g, 11-20g, 21-40g, 41-80g and >80g were recorded after harvesting. Data were pooled and analyzed statistically and means were separated according to the least significant differences (LSD) at 0.05 level of probability

## RESULTS AND DISCUSSION

### Emergence %

No significant variations were recorded in terms of emergence% among irrigation

methods and varieties. Among planting beds, significantly higher emergence% was recorded in Ridge and furrow planting (87.22) over flatbed planting method (83.89). Non significant variations were reported among interactions (Table 1). Higher emergence in ridge and furrow method might be due to the availability of more water in rows and their higher water holding capacity. It is because excess rainfall is properly directed through furrows. It also increases water-use efficiency

Table 1: Emergence and grade wise tuber number (000/ha) as influenced by irrigation method, planting method and variety

Treatments	Emergence %	Grade wise tuber number (000/ha)						% tuber by number		
		<3g	3-10g	11-20g	21-40g	41-80g	>80g	Total	<3g	41-80g
Irrigation method (A)										
Furrow	85.00	445	361	244	131	22	1.0	1204	36.8	1.7
Sprinkle	86.11	389	268	253	89	32	3.0	1034	37.4	3.0
SEm±	0.52	8.9	5.1	2.3	4.6	1.7	0.4	15.9	0.5	0.1
CD (P=0.05)	NS	55.0	31.7	NS	28.2	NS	NS	98.0	NS	0.7
Planting method (B)										
Flat Bed	83.89	450	316	241	86	30	0.0	1123	39.9	2.5
Ridge and furrow	87.22	384	313	256	134	24	4.0	1115	34.5	2.2
SEm±	1.06	7.8	7.3	4.5	4.3	1.6	0.5	10.6	0.6	0.1
CD (P=0.05)	3.27	24.1	NS	13.5	13.2	4.9	1.7	NS	1.7	NS
Variety (C)										
Kufri Lauvkar	86.81	437	334	232	143	43	2.0	1191	36.6	3.6
Kufri Surya	84.31	396	295	264	78	12	2.0	1047	37.7	1.1
SEm±	1.06	7.8	7.3	4.4	4.3	1.6	0.5	10.6	0.6	0.1
CD (P=0.05)	NS	24.1	22.5	13.5	13.2	4.9	NS	32.7	NS	0.4
Interaction A×B										
SEm±	1.50	11.1	10.3	6.2	6.1	2.2	0.8	15.0	0.8	0.2
CD (P=0.05)	NS	34.1	NS	19.0	18.7	NS	2.4	46.2	2.4	NS
Interaction A×C										
SEm±	1.50	11.1	10.3	6.2	6.1	2.2	0.8	15.0	0.6	0.2
CD (P=0.05)	NS	34.1	31.9	19.0	18.6	6.9	NS	NS	2.4	0.5
Interaction B×C										
SEm±	1.50	11.1	10.3	6.2	6.1	2.2	0.8	15.0	0.6	0.2
CD (P=0.05)	NS	34.1	31.9	19.0	18.6	6.9	NS	46.2	2.4	0.5
Interaction A×B×C										
SEm±	2.12	15.7	14.6	8.7	8.6	3.2	1.1	21.2	1.1	0.3
CD (P=0.05)	NS	48.3	NS	NS	NS	NS	NS	65.4	3.4	0.8

both under rainfed and irrigated scenarios because water moves laterally from furrows into beds thereby reducing evaporation losses (Chowdary *et al.*, 2022).

### Grade wise minituber number and weight (qha)

In the present study, <3g minitubers recorded significantly higher in furrow irrigation (445 thousand/ha and 7 q/ha), flat bed planting (450 thousand/ha and 7 q/ /ha)

and variety Kufri Lauvkar (437 thousand/ha and 7.1 q/ha) over sprinkler irrigation (389 thousand/ha and 5.6 q/ha), ridge and furrow planting (384 thousand/ha and 5.7 q/ha) and Kufri Surya (396 thousand/ha and 5.5 q/ha) respectively (table1 and 2). Interaction effect was found to be non significant. Similar results were reported in case of <5g minitubers in case of sprinkler irrigation study of Kufri Chandramukhi and Kufri Surya (Kaur *et al.*, 2019). Extremely small sized

Table 2: Grade wise tuber weight (q/ha) as influenced by irrigation method, planting method and variety

Treatments	Grade wise tuber weight (q/ha)						% tuber by weight		
	<3g	3-10g	11-20g	21-40g	41-80g	>80g	Total	<3g	41-80g
Irrigation method (A)									
Furrow	7.0	23.0	28.4	28.0	11.1	0.7	98.3	7.4	10.9
Sprinkle	5.6	17.7	35.8	29.1	14.7	2.7	105.7	5.3	12.6
SEm±	0.1	0.5	0.8	0.6	0.5	0.3	0.7	0.2	0.2
CD (P=0.05)	0.4	3.3	5.1	NS	2.4	1.6	4.4	1.1	1.2
Planting method (B)									
Flat Bed	7.0	20.0	32.1	24.1	13.0	0.0	96.2	7.4	11.9
Ridge and furrow	5.7	20.7	32.1	33.0	12.8	3.5	107.8	5.3	11.6
SEm±	0.1	0.6	0.9	0.9	0.6	0.4	1.0	0.1	0.5
CD (P=0.05)	0.3	NS	NS	2.8	NS	1.3	3.2	0.4	NS
Variety (C)									
Kufri Lauvkar	7.1	23.0	28.0	35.3	19.9	1.7	114.1	6.2	17.1
Kufri Surya	5.5	18.7	36.1	21.8	6.0	1.8	89.8	6.5	6.4
SEm±	0.1	0.6	0.9	0.9	0.5	0.4	1.0	0.1	0.5
CD (P=0.05)	0.3	1.8	2.8	2.8	1.6	NS	3.2	NS	1.5
Interaction A×B									
SEm±	0.1	0.8	1.3	1.3	0.7	0.6	1.5	0.2	0.7
CD (P=0.05)	NS	2.5	4.0	NS	2.3	1.8	4.5	NS	2.1
Interaction A×C									
SEm±	0.1	0.8	1.3	1.3	0.7	0.6	1.5	0.2	0.7
CD (P=0.05)	0.4	2.5	4.0	NS	2.3	NS	NS	0.6	2.1
Interaction B×C									
SEm±	0.1	0.8	1.3	1.3	0.7	0.6	1.5	0.2	0.7
CD (P=0.05)	0.4	2.5	NS	4.0	2.3	NS	4.5	0.6	2.1
Interaction A×B×C									
SEm±	0.2	1.2	1.8	1.8	1.0	0.8	2.1	0.3	0.9
CD (P=0.05)	NS	NS	NS	5.6	NS	NS	NS	0.9	NS

mini-tubers (<3g) were significantly affected by method of planting (Sharma *et al.*, 2014). The Under size Minituber category though of lower vigour is highly economic as it is first progeny of quality seed, and may be further be multiplied/ recycled (Kaur *et al.*, 2019). <3 g minituber/m<sup>2</sup> were maximum in Kufri Sindhuri and Kufri Bahar indicating varietal differences in extremely small size tuber production (Sadawarti *et al.*, 2018).

3-10g tubers were significantly higher in furrow irrigation method (361 thousand/ha and 23.0 q/ha) and Kufri Lauvkar (334 thousand/ha and 23.0 q/ha) over sprinkler irrigation (268 thousand/ha and 17.7 q/ha) and Kufri Surya (295 thousand/ha and 18.7 t/ha) respectively. No significant difference was recorded among planting bed type. 11-20g tubers were significantly higher in ridge and furrow planting method (256 thousand/ha) and Kufri Surya (264 thousand/ha and 36.1 q/ha) over flatbed method (241 thousand/ha) and Kufri Luvakar (232 thousand/ha and 28.0 q/ha) respectively. No significant difference was recorded among irrigation method in case of number of tubers. Reverse trend was reported in case of weight of minitubers in which sprinkler irrigation (35.8 q/ha) recorded significantly higher weight over furrow irrigation (28.4 q/ha). Furrow irrigation (131 thousand/ha), ridge and furrow planting method (134 thousand/ha) and variety Kufri Lauvkar (143 thousand/ha) recorded higher 21-40g grade tubers over sprinkler irrigation, flatbed planting and Kufri Surya respectively. Weight of tubers was significantly higher in ridge and furrow planting (33.0 q/ha) and Kufri Lauvkar (35.3 q/ha) over flatbed and Kufri Surya respectively (table 1 and 2).

Seed grade 41-80g tubers were significantly higher in flat bed planting method (30 thousand/ha) and variety Kufri Lauvkar (43 thousand/ha and 19.9 q/ha) over ridge and furrow bed (24 thousand/ha) and Kufri Surya

(12 thousand/ha and 6.0 q/ha) respectively. Sprinkler irrigation method (32 thousand/ha) recorded non significantly higher 41-80 g minitubers over furrow irrigation (22 thousand/ha) by number but by weight it was significantly higher over furrow irrigation (table 1 and 2). Seed size mini-tubers indicate the economically most important class of tubers with respect to seed multiplication and vigour. Varietal differences were significant with K. Surya producing higher number of seed sized tubers per square meter as compared to Kufri Chandramukhi for >30g minitubers (Kaur *et al.*, 2019). Over size >80g tubers recorded significantly higher minituber number in ridge and furrow method (4.0 thousand/ha) over flat bed system. In terms of weight >80g tubers grade was significantly higher in sprinkler irrigation (2.7 q/ha) over furrow irrigation (0.7 q/ha) and in ridge and furrow planting (3.5 q/ha) over flat bed system of planting. Large size mini-tubers (>20g) were significantly higher with flat bed method, whereas, 10-20g mini-tubers were almost same under both the methods of planting under microplant study (Sharma *et al.*, 2014).

### **Total minituber number and weight (q/ha)**

For total numbers of minituber, furrow irrigation (1204 thousand/ha) and Kufri Lauvkar (1191 thousand/ha) cultivar recorded significantly higher minitubers over sprinkler irrigation (1034 thousand/ha) and Kufri Surya (1047 thousand/ha). With regard to planting bed method flatbed method recorded non significantly higher total minitubers over ridge and furrow planting method. But interaction effects of irrigation× planting method× variety were significant (Table 1). Among varieties, Kufri Chipsona-1 recorded higher total tuber number in both, Gen-0 and Gen-1 over other two varieties under minituber multiplication study under Gwalior

conditions of India (Sadawarti *et al.*, 2021). Weight of tubers was significantly higher in sprinkler irrigation (105.7 q/ha), Ridge and furrow planting (107.8 q/ha) and variety Kufri Lauvkar (114.1) over furrow irrigation (98.3 q/ha), flat bed planting (96.2 q/ha) and variety Kufri Surya (89.8 q/ha) respectively (table 2). Advanced methods of irrigation such as sprinkler, micro sprinkler and drip produced higher tuber yield (21.87, 19.57 and 15.90 tonnes/ha respectively) as compared to conventional method of irrigation (Pawar and Dingre, 2014)) The treatments 30×10 cm under flat bed and ridge and furrow were at par for total number of tubers produced per square meter under aeroponic minituber multiplication study under net house (Kaur *et al.*, 2019). Under microplant multiplication study under two different spacing's, flatbed method of planting resulted in significantly higher number and yield of mini-tubers over the ridge furrow method (Sharma *et al.*, 2014). The significantly higher yield was obtained for the variety Kufri Surya as compared to variety Kufri Chandramukhi. (Kaur *et al.*, 2019). This also confirms present study wherein varietal differences were recorded. Significantly higher tuber yield (t/ha) was recorded in Kufri Lauvkar over Kufri Mohan and Kufri Chipsona-1 under minituber multiplication study (Sadawarti *et al.*, 2021). This indicate the varietal differences in terms of yield.

#### **Per cent extremely small (<3g) and seed size minitubers (40-80g)**

By number, only flatbed planting method (39.9%) recorded significantly higher <3g minitubers over ridge and furrow (34.5%). No significant difference was recorded among irrigation methods and cultivars (table1). By weight furrow irrigation and flat bed planting method (7.4%) recorded significantly higher <3g minitubers over sprinkler and ridge and furrow method of planting (5.3%). No

significant difference was recorded among cultivars (table 2). Varietal differences were reported for production of <3g minitubers (Sadawarti *et al.*, 2018). For seed size (40-80g), significantly higher seed size tubers were recorded in sprinkler irrigation method (3% by number and 12.6% by weight) and variety Kufri Lauvkar (3.6% by number and 17.1% by weight) over furrow irrigation and Kufri Surya. No significant but higher seed size tubers were reported both by number and weight in flat beds method over ridge and furrow method. Significantly higher > 20g minitubers were reported under flatbed method over ridge and furrow in microplant multiplication study under Kufi-Fagu conditions (Sharma *et al.*, 2014)

#### **CONCLUSION**

The study suggests that for higher production potential of aeroponically produced minitubers under nethouse, ridge and furrow method of planting along with sprinkler irrigation is suitable under North-Central conditions of India.

#### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

#### **ETHICAL STATEMENT**

This article does not contain any studies with human participants or animals performed by any of the authors

#### **LITERATURE CITED**

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# RESPONSE OF SOURCES AND LEVELS OF NITROGEN ON POTATO TUBER YIELD THROUGH DRIP FERTIGATION

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**ABSTRACT:** Potato growing in north Gujarat is most prevalent practice since last so many years. It is important *rabi* crop and gives good amount of money per unit area with best management practice. The experiment was undertaken during *rabi* 2019, 2020 and 2021 at Agricultural Research Station, S. D. Agricultural University, Aseda (Gujarat). The treatments comprised of three levels of source of nitrogen (S): S1 : Urea, S2 : 17-44 + micro nutrient grade III (1.0 %), S3 : WSF 19-19-19 and three levels of nitrogen (N) N1 : 100 % RDN, N2 : 75 % RDN, N3 : 50 % RDN. The experiment was laid out in a factorial randomized block design with three replications. Significantly higher tuber yield were recorded under the WSF 19-19-19 (40.08 t/ha) and 100% (RDN) treatment (37.75 t/ha). Interaction effect showed no significant result. Higher value of net return (₹ 186625/ha) and BCR of 3.00 were recorded under the treatment S1:Urea and 75% nitrogen (145032/ha, BCR of 2.20).

**KEYWORDS:** Source of fertilizers, Nnitrogen, Tuber yield, Dry matter, Starch

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of most important solanaceous vegetable crops which contributing to the world food requirements and occupied the fourth position after rice, wheat, and maize in production size (Walker *et al.*, 1999). The potato is most important tuber crops in India and grown worldwide. It is produced higher under Uttarpradesh state of about 15892 thousand tones with share of 29% of total country production followed by West Bengal (23.51%), Bihar (17.02%) and Gujarat (3780 thousand tones with shares of 7.05 % of total country production) as per report of NHB 2021-22. The area of potatoes harvested globally generally decreased from 2002 to 2020, before increasing again in 2021. The harvested area in 2021 was approximately 18.13 million hectares, an increase of over seven percent compared to the previous year. (Anonymus, 2023). Several reports compared the organic and synthetic fertilization of potato and revealed that best results are achieved by the last practice in terms of quantity and

quality of tubers (Palmer *et al.*, 2013; Singh and Lallawmkima, 2018). Overuse of the mineral fertilizers for long period not only makes soils degraded, polluted and less productive but have also posed severe health and environmental problems (Cockburn *et al.*, 2011). Drip irrigation is an effective way to supply water to the roots of plants and save water while maintaining a high yield and excellent product quality. It can easily be used for fertigation through which fertilizer is placed in the active root zone and crop requirements can be met accurately (Li *et al.* 2018). Fertigation also reduces the nutrient loss that would normally occur with the conventional methods of fertilizer application and thus, permits better availability and uptake of nutrients by the crops, leading to higher yield with high fertilizer use efficiency (Fan *et al.* 2020).

## MATERIALS AND METHODS

A field experiment was conducted consecutively for three years in *rabi* season

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of 2019, 2020 and 2021 at the Agricultural Research Station, S. D. Agricultural University on the fixed plots. Factorial Randomized complete block design with three replications were used to study the response of sources and levels of nitrogen on potato tuber yield through drip fertigation. The texture of the soil was loamy sand. The soil of the experimental field was low in available N (192 kg/ha), medium in available P (18 kg/ ha) and available K (190.0 kg/ha) and normal in soil reaction (pH 7.58) and electrical conductivity (0.2 mmhos/cm). The treatments comprised of three levels of source of nitrogen (S): S<sub>1</sub> : Urea, S<sub>2</sub> : 17-44 + micro nutrient grade III (1.0 %), S<sub>3</sub> : WSF 19-19-19 and three levels of nitrogen (N) N<sub>1</sub> : 100 % RDN, N<sub>2</sub> : 75 % RDN, N<sub>3</sub> : 50 % RDN. Source of nitrogen applied in five equal split at ten days Intervals in drip irrigation. Entire quantities of phosphorus and potash have been applied as basal in form of SSP and MOP in treatment S<sub>1</sub> and S<sub>2</sub>. Soil samples

were taken before treatments application and after completion of experiment to know the status of soil nutrient content and effect of nutrient imposition. The data were analyzed statistically by standard analysis of variance (ANOVA). Least significant difference (LSD) test was used to determine whether differences exist between certain comparisons. The probability level for determination of significance was 0.05.

## RESULTS AND DISCUSSION

### Effect of source and levels of nitrogen on growth and quality of potato

The results shown in table 1 indicated that the effect of different source and levels of nitrogen found non significant on emergence (%), plant height at 50 DAS and number of shoots per plant on pooled basis, However the significantly highest specific gravity starch and dry matter content was recorded in the treatment of S<sub>2</sub>: 17-44-00 as source of

Table 1: Effect of source and levels of nitrogen on growth, quality and yield of potato.

Treatments	Emergence (%)	Plant height (cm)	Number of shoots	Dry matter content (%)	Starch content (%)	Specific gravity (kg/m <sup>3</sup> )	Total tuber yield (t/ha)
S. Source of Nitrogen (S)							
S <sub>1</sub> : Urea	93.18	26.54	3.18	15.14	10.27	1.037	35.90
S <sub>2</sub> : 17-44 + micro nutrient grade III (1.0 %)	92.51	27.52	2.97	16.57	10.89	1.050	29.16
S <sub>3</sub> : WSF 19-19-19	92.07	28.47	3.34	15.66	10.32	1.044	40.08
SEm ±	0.47	1.08	0.12	0.09	0.07	0.001	0.77
CD at 5%	NS	NS	NS	0.27	0.19	0.003	2.21
N. Levels of Nitrogen (N)							
N <sub>1</sub> : 100 % RDN	92.66	28.97	3.27	16.28	10.71	1.048	37.75
N <sub>2</sub> : 75 % RDN	92.56	27.38	3.25	15.75	10.43	1.043	35.71
N <sub>3</sub> : 50 % RDN	92.51	26.18	2.96	15.34	10.35	1.039	31.68
SEm ±	0.47	0.57	0.07	0.09	0.07	0.001	0.77
CD at 5%	NS	1.61	NS	0.27	0.19	0.003	2.20
Interaction (S × N)							
S × N	NS	NS	NS	NS	NS	0.006	NS
Y <sub>x</sub> SXN	NS	NS	S.	NS	NS	NS	NS
C.V %	2.65	10.74	7.09	3.09	3.34	0.59	11.50

fertilizers (1.05,10.89 and 16.57 respectively) and 100 % RDN levels of nitrogen (1.048,10.71 and 16.28), while the lowest value found in the S1: urea and N<sub>3</sub>: 50% RDN The application of nitrogen through fertigation significantly improve the emergence and growth attributes was reported by Ghiyal and Bhatia 2018 and Yourtchi *et.al* (2013).

The result of interaction found non significant in case of starch and dry matter content while the specific gravity found significant and higher value was recorded under treatment S2N1 and remained at par with all other treatment except S1N2, S1N3 and S3N3. Fertigation with increased levels of nitrogen improve specific gravity of potato tubers reported by Jannat (2010), Kumar *et.al*. (2007), Jenkins and Nelson (1992).

Significantly highest value of specific gravity, starch and dry matter content was recorded in the treatment of S<sub>2</sub>: 17-44-00 as source of fertilizers and 100 % RDN levels of nitrogen.

### **Effect of sources and levels of nitrogen on total tuber yield and haulm yield**

The effect of different source and levels of nitrogen on total tuber yield and haulm yield found significant. In case of source of fertilizers, significantly highest total tuber yield (40.08 t/ha) was recorded under the WSF 19-19-19, while among different levels of nitrogen N1 : 100 % RDN recorded significantly higher value of total tuber (37.75t/ha) and remained statistically at par with the treatment N2 : 75 % RDN. The interaction effected remained non significant. Yield increased by the N rate. The soil N application gave higher yield than the zero N and lower than the fustigated treatment found by Mohammad et, al 1999. Seasonal (split) nitrogen management is proposed as a method to improve yield, quality and nitrogen fertilizer use efficiency of in determinant

cultivars reported by Ojala et. al. (1990), Ghiyal and Bhatia (2018), Jannat (2010), Fertigation resulted increased in crop yield by 20 to 30 % reported by Sandal *et al*. (2015) and Asrafi *et. al*. (2015), Sharma and Arora (2009), Kumar *et. al*. (2007), Walter *et.al* (2001), Jenkins and Nelson (1992) and Yourtchi *et.al* (2013).

The haulm yield (2702 kg/ha) was recorded significantly highest under the S3 : WSF 19-19-19 in different in case of source of fertilizers, significantly higher haulm yield (2466 kg/ha) was recorded under the N2 : 75 % RDN and remained statistically at par with the treatment N1 : 100 % RDN (2454 kg/ha) . The interaction effect of haulm yield due to different source and levels of nitrogen remained significant, the higher value was recorded under the treatment S3N2(2752kg/ha) and remained at par with the treatment S1N1 (2556kg/ha).

### **Effect of Source and levels of nitrogen on soil fertility status after harvest of crops**

Effect of different source on the PH, organic carbon content, available nitrogen content non significantly affected, while significant result has been recorded in case of available phosphorus and available potash status of soil after harvest of crop. Significantly higher value of available phosphorus and available potash were recorded under the treatment S3: WSF 19-19-19 while lower value was recorded under the treatment of urea. Effect of different levels of nitrogen on pH, OC and available nitrogen and available potash found non significant. Higher value of P<sub>2</sub>O<sub>5</sub> was recorded under the treatment of N3: 50 % RDN while lower value was recorded under the treatment N1 : 100 % RDN. The nutrient shows greater availability to crop and increase fertilizers use efficiency due to less leaching loss was reported by Jannat (2010). Asrafi *et.al*. (2015), Guler (2010), Kumar *et. al*. (2007) and Yourtchi *et.al* (2013).

Table 2. Economics of different treatments

Treatments	Tuber yield (t/ha)	Gross realization (₹/ha)	Cost of cultivation (₹/ha)	Net return (₹/ha)	BCR
S. Source of Nitrogen (S)					
S <sub>1</sub> : Urea	35.9	280035	93410	186625	3.00
S <sub>2</sub> : 17-44 + micro nutrient grade III (1.0 %)	29.16	228709	129771	98938	1.77
S <sub>3</sub> : WSF 19-19-19	40.08	313071	185112	127959	1.71
N. Levels of Nitrogen (N)					
N <sub>1</sub> : 100 % RDN	37.75	295032	154190	140842	2.14
N <sub>2</sub> : 75 % RDN	35.71	279516	134484	145032	2.2
N <sub>3</sub> : 50 % RDN	31.68	247267	119620	127647	2.13

Price of inputs (Rs. per kg): Urea@5.35, Potash@19, WSF 19-19-19@100, WSF 17-44-00@35, SSP@8, Tuber@7

## Economics

Among the different sources of fertilizers, S<sub>1</sub> recorded higher net realization of Rs. 186625/ha and 3.0 BCR value, while in case of levels of fertilizers S<sub>2</sub> (75% RDN) recorded higher net realization of Rs.145032/ha and 2.20 BCR value (Table 2). Net income and benefit cost ratio (B:C) indicated ultimately with the quality and yield which was higher with nitrogen fertilizers which conform with the observations of Kumar *et al.* (2007)

## CONCLUSION

For fetching the higher tuber yield of potato, when it has been cultivated through drip, it should be fertilized with 25% less nitrogenous fertilizer than the recommended fertilizer through urea in five equal split after 20 days after planting, while entire quantity of phosphorus and potash to be applied as basal at time of planting

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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# EVALUATION OF POTATO GERMPLASM FOR LATE BLIGHT AND POTATO CYST NEMATODE RESISTANCE UNDER NORTH-WESTERN INDIAN HILLS

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**ABSTRACT:** Late blight disease caused by the fungus *Phytophthora infestans* is the main yield limiting factor amongst the diseases of potato in India and across the globe. Potato Cyst Nematode/PCN (*Globodera* spp.) is another biotic factor of economic importance and is a quarantine organism. Exploitation of genetic resistance is the most preferred management strategy. In the present study, indigenous potato genotypes collected from different parts of the country along with checks were evaluated for their resistance against late blight under natural epiphytotic field conditions and in laboratory under artificially inoculated conditions including detached leaf and tuber slice assays and PCN through Root ball technique during 2020-2023. The converted field scale values i.e. average AUDPC value ranged from 7 in highly resistant genotype Rangpuria to 1596 in susceptible accession AGR/56. The field blight response was comparatively more correlated ( $r = 0.63$ ) with susceptibility levels measured in the detached leaf assay compared to that with tuber slice assay ( $r = 0.48$ ). Genotypes Kanpuria Safed, JG-1, Rangpuria and Desi Aloo showed resistance to late blight both under laboratory and field conditions. For PCN, Garlentic, Jeevan Jyoti and JG-1 have combined resistance (0 female/root ball) over the years to both the species. Majority of the studied genotypes have medium to high pollen viability with desirable tuber traits and yield advantage. The identified resistant genotypes excel their usage as parental lines in breeding for biotic stress resistance specifically for late blight and PCN.

**KEYWORDS:** Late blight, PCN, Yield, Indigenous potato germplasm

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is presently the most important non-grained food crop that was introduced in the early 17<sup>th</sup> century. Since its introduction as a garden vegetable in Western India, potato farming extended throughout India over the next two and a half centuries. *S. tuberosum* ssp. *andigena* was probably the first potato species introduced in India. These initial introductions later on got adapted and are known by varied names in different dialects/languages creating uncertainty about their identification and nomenclature. Potato Synonym Committee, National Institute of Agricultural Botany, England based on morphological examinations

culled duplicate samples and initially identified 16 non-European varieties known as *desi* or indigenous samples or varieties that are result of survivors of earlier introductions and chance selections in the Indian agro-climates. Pal and Pushkarnath 1951 testified bud mutation can also be the origin of these *desi*/indigenous potato varieties. *Desi* varieties have salient features of tolerance to abiotic stresses viz., heat and drought and to degenerative viruses, shorter dormancy, early maturity and preferred culinary and quality parameters. Slower rate of degeneration benefits potato growers by lesser need of seed replacement.

The collection of *desi* potato germplasm repository at CPRI Shimla is strengthened by

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explorations in different parts of the country and present repository constitutes more than 100 indigenous accessions conserved *in vitro* at Shimla and *in vivo* at Kufri.

Late blight and PCN are one of the two major biotic factors hindering potato cultivation in north India hills constituting parts of Himachal Pradesh, Uttarakhand and Jammu & Kashmir. Late blight, a fungal infection caused by *Phytophthora infestans* (Mont.) de Bary, causes significant yield losses leading to high levels of fungicidal usage across all potato growing conditions. Emergence of new virulent pathotypes necessitates search of new chemical formulation as well as resistance sources to manage this pathogen. Host resistance is the most environment-friendly, cost effective, durable and consumer health friendly way of disease management and late blight over fungicidal applications. Search of new resistance genes in cultivated as well as wild potato species and interspecific somatic hybrids are being done (Bhatia *et al.* 2023) to broaden the genetic base for blight resistance.

PCN, *Globodera rostochiensis* and *G. pallida* are important pests feeding on potato roots causing major losses up to 30%. In India both species are prevalent as mixed populations in all potato growing areas of southern hills and recent interception in north-western hills also confirm presence of both species. Like late blight, host resistance is durable method of managing PCN populations. Cultivated and wild species viz., *S. tuberosum* ssp. *andigena*, *S. vernei*, *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii* are mainly used in PCN resistance breeding (Dalamu *et al.* 2012).

In lieu for above information, the present study was undertaken for search of novel late blight and PCN resistant genotypes in a set of *desi* potato genotypes that have not been studied up to now and to identify their potential usage as parental lines in resistance breeding based on yield, maturity and floral parameters.

## MATERIALS AND METHODS

### Late blight-Screening under natural epiphytotic field condition

Thirty-eight potato genotypes were evaluated for late blight resistance in replicated, single row trials natural epiphytotic conditions at Kufri Hills (Figure 1a) (2743 m amsl; 31.10°N, 77.25°E) in summer season of 2021, 2022 & 2023 including 35 test accessions (*desi* genotypes) and standard controls namely Kufri Girdhari (highly resistant), Kufri Himalini (moderately resistant) and Kufri Jyoti (susceptible). Data on disease severity was recorded at an interval of seven days between two subsequent readings, and the area under the disease progress curve (AUDPC) was calculated (Shaner and Finney 1977). Based on AUDPC, the genotypes were graded as highly resistant/HR (< 250); resistant/R (251-350); moderately resistant/MR (351-650) and susceptible/S (> 650).

### Late blight-Screening under Laboratory condition- Detached leaf and Tuber slice assays

Late blight reaction was reconfirmed by laboratory analysis by detached leaf assay (Singh *et al.* 1997) through challenge inoculation with complex races of *Phytophthora infestans* (Figure 1b). On an average, six leaflets of top 4-5<sup>th</sup> leaf from 2-4 plants of 45 days old were artificially inoculated by complex races of *P. infestans* using  $6 \times 10^4$  sporangia/ml. On the basis of lesion area (cm<sup>2</sup>), the genotypes are classified as highly resistant (lesion area up to 1.0 cm<sup>2</sup>); resistant (1.1 to 2.5 cm<sup>2</sup>); moderately resistant (2.51-5.0 cm<sup>2</sup>) and susceptible (>5.0 cm<sup>2</sup>). In Tuber slice assay, tuber slices (1 cm thick) in triplicate of each genotype placed in sterilized petri dishes are inoculated with zoospore suspension using filter paper discs (0.3 cm<sup>2</sup>) dipped in the zoospore suspension ( $6 \times 10^4$  sporangia/ml). The genotypes are categorised as per scale given under foliage resistance by detach leaf assay.

### PCN-Screening by Root ball assay

Meanwhile these accessions were also evaluated for PCN resistance through Root ball technique (Figure 1c) under glass house conditions with controls SM/11-120 (highly resistant) and Kufri Jyoti (highly susceptible). Five tubers of each genotype were planted in approx. 10 cm diameter pots in a glass house at 20-22 °C. The planting soil had a population of 200-250 cysts of both *Globodera* species per 100 mg, resulting in 8000-10000 eggs and larvae per test tuber. The number of females on the root balls of various cultures was counted after 60-65 days, when the females were evident on the root balls of the control plants (Kufri Jyoti). Grade 0-1 clones are the most desired level of resistance (Krishna Prasad 2006). The colour of developing females distinguishes the two species of *Globodera*. The genotypes were graded based on cyst count as highly resistant (0 female/root ball); resistant (1-5 female/root ball); moderately resistant (6-20 female/root ball); susceptible (21-50 female/root ball) and highly susceptible (>50 female/root ball).

### Pollen viability

Freshly opened flowers or buds about to open (Balloon stage) are collected and stored in paper bags. The pollen samples were stained using acetocarmine glycerol. A small amount of pollen is mixed with an acetocarmine glycerol jelly drop placed in the

centre of the slide. A slide cover was kept after a minute, and the mounted slides were checked for pollen staining. Pollen grains with full staining, plumpness, and clearly defined outlines were regarded as viable, whereas those without staining or with irregular shapes are non viable. Data recorded in four replicates are expressed as the percentage of the viable pollens. The genotypes are graded as highly fertile >70%; medium fertile 40-70%; low fertile 10-40%; sterile <10%.

### RESULTS AND DISCUSSION

Among various biotic stresses of potatoes, late blight is one the most devastating affecting both foliage and tuber thus reducing tuber yield as well as tuber quality. It is widespread in both hills and sub-tropical regions of the country. Though initially considered disease of temperate world (Kumar *et al.* 2007), now the disease is also prevalent in the sub-tropical plains mandating development of at least moderately resistant varieties for these areas.

In the present study resistance responses of genotypes were compared by field evaluations and laboratory methods of detached and tuber slice assays. The majority of genotypes showed differential reaction within the field and lab assays.

In the natural epiphytotic field conditions, three genotypes *viz.*, Kanpuria Safed, Rangpuria and Desi also had average AUDPC values of



Fig.1. Field (a) and Detached leaf assay (b) for late blight; Root ball assay for PCN (c)

years 2021, 2022 and 2023 lower than 250 and grouped as highly resistant to late blight (Table 1). Only accession JG-1 was resistant (AUDPC 251-350) while three accessions, Australian White, G-4 and NJ-62 were moderately resistant (AUDPC 351-650). Majority (80%) of accessions were categorised as susceptible. The

results corroborate with earlier evaluations of native potato genotypes for two seasons under natural epiphytotic conditions with minor difference of some accession previously found highly resistant are now resistant and vice versa (Dalamu *et al.* 2023). Late blight appears every year in epiphytotic form at hills in potato

**Table 1. Potato genotypes and their late blight response under laboratory and natural epiphytotic field condition and PCN through Root ball assay during 2020 -2023**

Genotypes	Late blight Reaction							PCN Reaction			
	Laboratory		Field condition (AUDPC)					Root ball assay			
	Detached leaf assay	Tuber slice assay	Natural epiphytotic condition (hot spot)					GRO/ GPA			
			2022	2021	2022	2023	Average	Disease reaction	2020	2021	2022
AGR/56	R	MR	1481	1595	1712	1596	S	S/HS	S/HS	S/HS	S/HS
Aruconia	R	MR	862	1241	996	1033	S	S/ S	S/ S	S/S	S/ S
Australian White	R	MR	574	608	583	588	MR	HS/HS	HS/HS	HS/HS	S/ S
Beeta	R	R	780	810	930	840	S	HS/ S	HS/ S	HS/ S	S/ S
Bengal Jyoti	S	MR	1582	1280	1571	1478	S	HS/ S	HS/ S	HS/ S	HS/ S
Bhura Aloo/Alu	S	MR	1357	1394	1300	1350	S	HS/ S	HS/ S	S/ S	S/ S
Dehati Aloo/ Alu	S	R	775	717	765	752	S	HS/ MR	S/MR	HS/ MR	HS/MR
Desi Aloo/ Alu	HR	R	251	255	102	203	HR	HS/ S	HS/ S	HS/ S	S/ S
DRR Blue	S	R	1501	1396	1282	1393	S	HS/HS	HS/HS	HS/HS	HS/HS
Deshla Lal	S	R	1568	1469	1685	1574	S	S/ S	S/ S	HS / S	S/ S
Garlentic	R	R	1479	1469	1685	1544	S	HR/ HR	HR/ HR	HR/ HR	HR/ HR
G-4	MR	R	461	500	478	480	MR	S/ S	HS/ S	HS/ S	S/ S
Hamraj Hatti	S	R	1268	1281	1256	1268	S	S/ MR	S/MR	S/MR	S/ MR
Jeevan Jyoti	S	S	1405	1468	1309	1394	S	HR/ HR	HR/ HR	HR/ HR	HR/ HR
JG-1	R	R	244	240	268	251	R	HR/ HR	HR/ HR	HR/ HR	HR/ HR
JG-22	S	MR	1180	944	980	1035	S	S/S	HS/S	HS/S	S/S
Kanpuria Safed	HR	R	12	17	41	23	HR	HS/HS	HS/S	S/S	S/S
Kacha Bhutia	MR	R	1614	1464	1512	1530	S	S/S	S/S	S/HS	S/S
K-22	R	S	1500	1569	1545	1538	S	HS/HS	HS/HS	HS/HS	HS/ HS
KP/PC-292	R	R	1280	1281	1274	1278	S	HS/MR	S/MR	S/MR	S/MR
Lal Ankh	S	S	1303	1298	1298	1300	S	HS/HS	HS/ HS	HS/ HS	HS/ HS
Lal Mitti-1	R	R	1486	1304	1571	1454	S	HS/ HS	HS/HS	HS/ HS	HS/ HS
Lal Mitti-2	S	R	1528	1383	1476	1462	S	HS/ HS	HS/ HS	HS/ HS	HS/ HS
Lal Gulab	S	MR	1080	947	980	1002	S	HS /MR	S/MR	S/ S	S/ S
Lah Saw Smit	S	S	1521	1566	1592	1560	S	HS/ S	HS/ S	HS/ S	HS/ S
NJ-12	S	S	898	920	973	930	S	HS/ HS	HS/HS	HS/HS	HS/ HS

Genotypes	Late blight Reaction							PCN Reaction			
	Laboratory		Field condition (AUDPC)					Root ball assay			
	Detached leaf assay	Tuber slice assay	Natural epiphytotic condition (hot spot)					GRO/ GPA			
			2022	2021	2022	2023	Average	Disease reaction	2020	2021	2022
NJ-47	S	S	1170	941	980	1030	S	S/MR	S / MR	S/ MR	S/ MR
NJ-62	S	R	624	608	597	610	MR	S/MR	S/MR	S/ S	S/ S
NJ-78	S	S	1413	1368	1309	1363	S	S/ S	S/ S	S/ S	S/ S
NJ-130	S	S	1138	1044	1008	1163	S	HS/ S	HS/ S	HS/ S	HS/ S
PSK-76	MR	S	1207	1255	1244	1235	S	HS/HS	HS/HS	HS/ HS	HS/ HS
Phulwa Red	S	S	1314	1344	1317	1325	S	MR/ R	MR/ R	MR/ R	MR/ R
Rangpuria	HR	R	5	7	8	7	HR	S/HS	S/HS	S/HS	S/HS
VB-8	R	S	1380	1365	1309	1351	S	S/S	S/S	S/S	S/S
V2-2912	S	S	842	901	934	892	S	HS/S	HS/S	HS/S	HS/S
Kufri Jyoti (Control)	S	S	1200	1215	1212	1209	S	HS/ HS	HS/ HS	HS/ HS	HS/ HS
Kufri Girdhari (Control)	-	-	7	11	20	13	HR	-	-	-	-
Kufri Himalini (Control)	-	-	423	398	418	413	MR	-	-	-	-
SM/11-120 (Control)	-	-	-	-	-	-	-	HR/ HR	HR/ HR	HR/ HR	HR/ HR

GRO: *G. rostochiensis*; GPA: *G. pallida*

and other solanaceous crop growing regions as well as in kharif potato season compared to lower intensity in plains. Low temperature (16-20°C) with high relative humidity (>70%) are congenial for initiation of disease infection and further spread. In detached leaf assay, three accessions, Desi aloo, Kanpuria Safed and Rangpuria were highly resistant (lesion area up to 1.0 cm<sup>2</sup>) while none genotype in tuber slice assay. The reaction pattern of some accession was similar in both field and laboratory assays and within two lab methods while others had differential responses under varying screening methodologies.

### Correlation of late blight reaction in field condition versus laboratory assays

The genotypes were grouped as resistant and susceptible based on all the three methods of late blight evaluations. The field assay of foliar blight resistance under natural

epiphytotic condition had higher association with detached leaf assay ( $r=0.63$ ) compared to that with tuber slice assay ( $r=0.48$ ) that may be attributed to difference in plant parts used in resistance screening. Similar results are reported by Srivastava *et al.* 2015 in a group of exotic potato germplasm evaluated under field and detached leaf techniques where up to 64 percent accessions reported similar reaction. The differences may be due to variations in foliage vs tuber infection, age and maturity of tuber, storage conditions, canopy coverage, spatial and temporal variations in pathogen pressure etc. Inconsistencies in field versus laboratory method of blight screening have also been previously reported (Douches *et al.* 2002) where high levels of resistance in lab assays were not found in the field necessitating both method of screening for confirmation of resistance and laboratory assays cannot substitute evaluation under

field conditions.

PCN populations are present in mixed form in infected soil necessitating search of genotypes bearing resistance to both species. Three genotypes, Garlentic, Jeevan Jyoti and JG-1 were highly resistant (0 female/root ball) to both white and pale cyst nematodes (**Table 1**). Only Phulwa Red was moderately resistant (6-20 female/root ball) to *G. rostochiensis* and resistant to *G. pallida*. Accessions, Dehati Aloo, Hamraj Hatti, KP/PC-292 and NJ-47 were moderately resistant to *G. pallida* only. Majority of the genotypes (80%) were either susceptible or highly susceptible to both the PCN species. However, Sudha *et al.* 2019 reported 67% genotypes (44 out of 66) evaluated at Southern hills resistant to both the species of PCN. Such high number of resistant lines in that study is due to inclusion of mainly parental lines and advanced selections where PCN was intercepted long ago in 1960s and resistance breeding including development of parental lines was undertaken in mission mode.

### Pollen viability

Pollen viability determines potential usage of genotypes as parental lines and thereby success in breeding activities. Evaluation of pollen viability revealed 20 genotypes had moderate to high pollen viability (**Table 2**). Eight genotypes showed unviable pollen or sterile pollen. Flowering was profuse in seven genotypes, medium in thirteen and fifteen genotypes had sparse flowering. DRR Blue, Deshla Lal, G-4, Kacha Bhutia and NJ-62 bear profuse flowering with high pollen viability. Meanwhile genotypes with beneficial traits i.e late blight and PCN resistance, Desi aloo, Garlentic, Jeevan Jyoti, JG-1 and Rangpuria, though had sparse to medium flowering but pollen viability is high. Sudha *et al.* 2019 described 86% lines constituting potato parental lines, wild *Solanum* species, advanced

**Table 2. Morphological features and yield performance of potato genotypes**

Genotypes	Source	TTY (q/ha)*	MD (days)†	Canopy	PH (cm) **	Tuber skin colour	Flesh colour	Tuber shape	Eye depth	PMD®	Flowering intensity	PV‡
AGR/56	Kashmir, J&K	110.00	Early	Semi-compact	Medium	Yellow	Cream	Ovoid	Medium deep	Absent	Medium	Sterile
Aruconia	Not available	258.30	Medium	Semi-compact	Tall	Whitish cream	Cream	Round	Shallow	Absent	Sparse	Sterile
Australian White	Midnapore, WB	121.40	Medium	Semi-compact	Medium	Brown	Yellow	Ovoid	Shallow	Present	Sparse	High
Beeta	Gangtok, Sikkim	129.10	Medium	Semi-compact	Medium	Whitish cream	Cream	Round	Shallow	Absent	Profuse	Medium
Bengal Jyoti	Nalanda, Bihar	475.00	Late	Compact	Medium	Whitish cream	Yellow	Oblong	Shallow	Absent	Medium	High
Bhura Aloo	Dholi, Bihar	390.00	Medium	Compact	Medium	Red	Cream	Round	Deep	Present	Sparse	Low
DehatiAloo	Patna , Bihar	152.00	Early	Open	Medium	Whitish cream	White	Ovoid	Shallow	Absent	Medium	High
Desi Aloo	Chamba, HP	354.00	Late	Semi-compact	Medium	Yellow	Yellow	Round	Shallow	Absent	Medium	High
DRR Blue	Allahabad, UP	236.20	Late	Open	Medium	Purple	Yellow	Round	Shallow	Absent	Profuse	High
Deshla Lal	Nalanda, Bihar	254.40	Early	Semi-compact	Medium	Red	Yellow	Round	Medium deep	Absent	Profuse	High
Garlentic	Kanpur, UP	309.00	Medium	Open	Small	Whitish cream	Yellow	Round	Shallow	Present	Sparse	High

Genotypes	Source	TTY (q/ha) <sup>#</sup>	MD (days) <sup>*</sup>	Canopy	PH (cm)	Tuber colour	Flesh colour	Tuber shape	Eye depth	PMD <sup>@</sup>	Flowering intensity	PV <sup>\$</sup>
G-4	Farrukhabad, UP	386.80	Early	Semi-compact	Medium	Whitish cream	Cream	Oblong	Shallow	Absent	Profuse	High
Hamraj Hatti	Nalanda, Bihar	338.30	Medium	Compact	Medium	Red	Cream	Round	Medium deep	Absent	Sparse	Low
Jeevan Jyoti	Burdwan, WB	319.50	Early	Semi-compact	Medium	Red	Yellow	Round	Shallow	Present	Sparse	High
JG-1	East Khasi hills, Meghalaya	245.00	Medium	Semi-compact	Small	Whitish cream	Cream	Round	Shallow	Absent	Sparse	High
JG-22	Jammu & Kashmir	249.00	Medium	Compact	Small	Whitish cream	Cream	Ovoid	Medium deep	Absent	Medium	Low
Kanpuria Safed	Kanpur, UP	326.30	Medium	Compact	Medium	Yellow	Cream	Ovoid	Shallow	Present	Sparse	Low
Kacha Bhutia	Gangtok, Sikkim	414.30	Medium	Semi-compact	Medium	Whitish cream	White	Ovoid	Medium deep	Absent	Profuse	High
K-22	Midnapore, WB	148.00	Early	Compact	Medium	Whitish cream	White	Ovoid	Shallow	Absent	Medium	Low
KP/PC-292	Nakodar, Punjab	243.00	Early	Compact	Medium	Orange	White	Round	Shallow	Absent	Sparse	Sterile
Lal Ankh	Jorhat, Assam	305.20	Early	Semi-compact	Medium	Red	Cream	Round	Protruding	Absent	Sparse	Sterile
Lal Mitti-1	Nalanda, Bihar	243.20	Late	Semi-compact	Medium	Red	Yellow	Ovoid	Shallow	Absent	Medium	High
Lal Mitti-2	Nalanda, Bihar	263.00	Medium	Open	Medium	Red	Yellow	Ovoid	Shallow	Present	Sparse	Medium
Lal Gulab	Farrukhabad, UP	466.00	Medium	Compact	Medium	Red	Cream	Flattened	Shallow	Absent	Medium	High
Lah Saw Smit	Shillong, Meghalaya	277.30	Medium	Compact	Small	Whitish cream	Cream	Round	Medium deep	Present	Sparse	Medium
NJ-12	Kinnaur, HP	150.00	Medium	Compact	Tall	Whitish cream	Cream	Round	Medium deep	Absent	Sparse	Low
NJ-47	Kinnaur, HP	182.00	Medium	Open	Small	Whitish cream	Cream	Round	Medium deep	Absent	Sparse	Sterile
NJ-62	Chamba, HP	114.00	Medium	Compact	Medium	Whitish cream	Cream	Round	Round	Absent	Profuse	High
NJ-78	Chamba, HP	73.00	Medium	Semi-compact	Medium	Whitish cream	Yellow	Ovoid	Medium deep	Absent	Medium	Sterile
NJ-130	Kinnaur, HP	140.00	Medium	Semi-compact	Medium	Whitish cream	White	Ovoid	Round	Absent	Medium	Sterile
PSK-76	Basti, UP	243.70	Medium	Semi-compact	Medium	Whitish cream	White	Ovoid	Shallow	Absent	Medium	Low
Phulwa Red	Jorhat, Assam	294.50	Early	Semi-compact	Medium	Red	Yellow	Round	Shallow	Absent	Sparse	High
Rangpuria	Cooch Behar, WB	321.00	Late	Semi-compact	Tall	Whitish cream	Yellow	Ovoid	Medium deep	Absent	Medium	High
VB-8	Shimla, HP	320.00	Early	Semi-compact	Small	Whitish cream	White	Round	Shallow	Absent	Medium	High
V2-2912	Shimla, HP	292.00	Early	Open	Medium	Whitish cream	White	Ovoid	Medium deep	Absent	Profuse	Sterile

# TTY: Total tuber yield-Average of three years (2017, 2018 & 2019); \*MD:Maturity duration-Early < 80 days; Medium 80-100 days; Late >100 days; \*\*PH:Plant height -Small <50cm; Medium 50- 70cm; Tall >70cm; @PMD:Premature bud dropping;\$PV:Pollen viability-High>70%; Medium 40-70%; Low 10-40%; Sterile<10%

selections and cultivars with medium to high pollen viability. Additionally, only seven genotypes presented pre mature dropping of floral buds that is the major cause of sterility in potato.

Genotypes Desi aloo, JG-1, Kanpuria Safed and Rangpuria had consistent resistance to late blight across the seasons and method of evaluations. Garlentic, Jeevan Jyoti and JG-1 had steady resistance response to both species of *Globodera* in all the four years of evaluations. Additionally, these genotypes have high total tuber yield (average 312 q/ha), plant maturity is early to mid to late with semi-compact canopy. Economic part, tubers are round to ovoid, tuber skin colour range from whitish cream to yellow to red and tuber flesh varied from cream to yellow with shallow eyes. High pollen fertility in majority of genotypes facilitates their usage as parental lines in breeding for combined resistance to late blight and PCN with early to medium maturation.

## CONCLUSION

Late blight and PCN are the two most dreaded biotic stresses of potato. Resistance breeding requires constant attempt to stay ahead of the rapidly evolving pathogen signifying search of new resistance genes with robust phenotypic screening. Identified late blight and PCN resistant genotypes Kanpuria Safed, JG-1, Rangpuria, Desi Aloo, Garlentic and Jeevan Jyoti would augment the Indian potato resistance breeding activity.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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## EVALUATION OF AEROPONIC MINITUBER'S POTENTIAL FOR BASIC SEED PRODUCTION

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**ABSTRACT:** The study conducted at ICAR-Central Potato Research Institute, Regional Station Gwalior over a six-year period from 2017-18 to 2022-23 aimed to evaluate the performance of 17 popular potato varieties. These varieties were divided into early maturing and medium maturing categories. The study focused on various yield attributes and health parameters of these potato varieties, with a particular emphasis on breeder seed production. The emergence percentage ranged from 85% to 99%, indicating that different varieties had varying rates of successful sprouting and growth. Health status of the varieties was found sound. Variability was observed among varieties for yield attributing parameters and Kufri Neelkanth emerged as the top-performing variety in the study. It displayed the highest seed yield per square meter (3.31 kg), the highest number of tubers per square meter (231), and the largest mean seed weight (25.1 grams). Additionally, it had a significant seed multiplication ratio (SMR) by both number (6.9) and weight (25.1).

**KEYWORDS:** Healthy planting material, aeroponics, minitubers, varieties, virus incidence

Aeroponic systems for potato pre-basic seed production were established following increased demand for more efficient, high quality seed production (Ritter *et al.* 2001, Nichols 2005) and has taken its feet at the turn of the century, as an efficient, high-quality potato minituber production technology. It is an alternative technique of soilless culture in growth-controlled environments (Singh *et al.*, 2020) and can produce higher yields (up to 10-times higher), more quickly, and at lesser cost than conventional growing methods (Devi *et al.*, 2019). Aeroponics system has maximum application in potato seed production (Ritter *et al.*, 2001; Factor *et al.*, 2007). Owing to higher rates of multiplication as well as improved health standards and size regulation with sequential harvesting of minitubers favoring potato seed growers (Farran and Mingo-Castel 2006). As of now aeroponic system has become an integral part of potato

seed production across the world, including India (Buckseth *et al.*, 2016). Mini-tubers are principally used for the production of pre-basic or basic seed by direct field planting (Ritter, 2001). Efficient management and utilization of these small sized minitubers to produce successive disease free generations is the next most critical issue after the successful advent of the aeroponic technology. Being a nascent technology, there is a severe dearth knowledge related to the management and planting of the aeroponic minitubers (Devi *et al.*, 2019). Cultivars differ widely in their capacity to produce minitubers, some have high and some have low yielding potentials (Caligari and Powell, 1989). Sometimes the potentiality of newly developed varieties is not known in terms of all categories of tubers. However, seed minituber is the first generation seed; it is important to produce sufficient qualities of minituber. The variability

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of multiplication rates of different varieties should be determined before going to plan any seed production scheme (Mallick *et al*, 2017). In India, at the time of standardization of Seed Plot Technique in late sixties, the virus profile comprised of only six viruses (PVS, PVX, PVA, PVM, PVY, PLRV). New strains of PVY (PVY<sup>n</sup>, PVY<sup>o+c</sup>) have either been introduced or developed through mutations (Kreuze *et al*, 2020; Singh and Sharma, 2018). Today, virus profile is much wider and complex than what it was in seventies when seed plot technique was developed (Singh and Sharma, 2018). ELISA testing consists of detecting the antigen-antibody interactions by enzyme induced color reaction which is reasonably sensitive and highly amenable to high throughput automation hence suitable for large scale screening of samples. With this background, first field performance of the precious delicate aeroponic minitubers of different early and medium maturing varieties were evaluated for its production potential and health standards thereof.

The aeroponic minitubers of potato varieties were produced in aeroponic facility at ICAR-CPRI, Shimla which were further planted during crop season of 2017-18 to 2022-23 (for 6 years) under aphid proof net house conditions at ICAR Central Potato Research Institute, Regional Station Gwalior. Aeroponic minitubers of 17 varieties viz., K. Chandramukhi (KCM), K. Lauvkar, K. Surya, K. Pukhraj, K. Khyati, K. Lima (early maturing ) and K. Jyoti, K. Chipsona-1, K. Chipsona-3, K. Himalini, K. Mohan, K. Karan, K. Ganga, K. Thar.-2, K. Sangam, Kufri Neelkanth and K. Garima (medium maturing ) under seed production system for pre-basic seed production were planted/ multiplied and taken under study. Details of planting years, variety wise average area and number of aeroponic minitubers planted is given in table 1. Aeroponic minitubers weighing ~4-5g were

planted on well prepared beds during last week of October to second week of November in different years. Seed bed was prepared after addition of one-inch-thick layer of well decomposed farm yard manure and sand in the ratio of 1:1 on the top of the bed. Well sprouted aeroponic minitubers were planted at inter and intra row spacing of 30×10 cm in 2 m wide stripas per availability in different years. A light irrigation was given with the help of rose cane just after planting and subsequent irrigation was given as per the requirement and other packages of practices were followed as per seed plot technique. After 85-90 days after planting, haulms were pulled manually and after 10-12 days of de-haulming the crop was harvested manually. The data recorded during the study are plant emergence % (25-30 DAP), mean tuber weight (g), seed multiplication rate both by number and weight (formulae) , tuber yield both by number and weight per m<sup>2</sup> at the time of harvesting and mean was calculated. Health standard for major viruses viz PVX, PVS, PVY, PVM, PVA, PLRV, PVY<sup>n</sup> and PVY<sup>o+c</sup> was evaluated with Enzyme-linked Immuno Sorbent Assay (ELISA) test.

Seed multiplication rate both by number and weight = No. and weight of tuber produced per M<sup>2</sup>/ No. and weight of tuber planted per M<sup>2</sup>

Emergence % varied among the varieties and it ranged from 85-99 %. Highest emergence was reported in K. Lima (99.50%) and lowest in K. Chandramukhi (85%) table 1. Variability in terms of emergence was also reported among varieties in microplant, microtuber and recycled minituber study (Sadawarti *et al*, 2018) and aeroponic minituber multiplication study (Sadawarti *et al*, 2021). The percent survival/establishment was significantly influenced by potato cultivar and type of planting material and their interaction under *in vivo* conditions (Venkatasalam *et al.*, 2011). This confirms the present study.

**Table 1: Emergence and yield parameters of different varieties under field multiplication of aeroponic minitubers**

Variety	No. of year planted	Average						Seed multiplication Rate	
		Number Planted	Area planted (m <sup>2</sup> )	Emergence %	Yield (Kg/m <sup>2</sup> )	Yield (No/m <sup>2</sup> )	Tuber weight (g)	By number	By weight
KCM	1	2910	87.3	85.00	1.15	110	10.4	3.3	8.7
K. Lauvkar	2	8128	243.8	89.50	1.72	146	11.8	4.4	13.0
K. Jyoti	2	1377	41.3	97.00	1.69	135	12.5	4.1	12.8
K.Chipsona-1	5	2117	63.5	95.00	1.20	156	7.7	4.7	9.1
K.Chipsona-3	2	357	10.7	99.00	1.17	136	8.6	4.1	8.8
K. Surya	2	1265	38.0	97.50	1.15	126	9.1	3.8	8.7
K. Pukhraj	4	1456	43.7	95.25	1.27	183	7.0	5.5	9.6
K. Khyati	4	2448	73.4	92.50	1.06	118	8.9	3.5	8.0
K. Lima	2	1908	57.2	99.50	1.53	98	15.6	2.9	11.6
K. Himalini	2	2577	77.3	97.00	1.82	108	16.9	3.2	13.8
K. Mohan	4	3338	100.1	96.13	1.58	141	11.2	4.2	12.0
K. Karan	2	700	21.0	96.00	2.40	120	20.0	3.6	18.2
K. Ganga	2	2273	68.2	98.00	2.68	214	12.5	6.4	20.3
K. Thar.- 2	1	777	23.3	95.00	0.86	101	8.5	3.0	6.5
K. Sangam	1	1137	34.1	98.00	1.47	133	11.0	4.0	11.1
K. Neelkanth	1	2013	60.4	97.00	3.31	231	14.4	6.9	25.1
K. Garima	1	3200	96.0	94.00	0.49	145	3.4	4.4	3.7

Variability was reported in terms of yield parameters among varieties. Tuber yield/m<sup>2</sup> was ranged from 0.49-3.31 kg and highest was reported in K. Neelkanth (3.31Kg/m<sup>2</sup>) and lowest in K. Garima (0.49 Kg/m<sup>2</sup>). Highest tuber number/ m<sup>2</sup>were recorded in K. Neelkanth (231) followed by K. Ganga (214) and lowest in K. Lima (98). Highest mean tuber weight was reported K. Karan (20.0g) and lowest in K. Garima (3.4g). Seed multiplication rate by number was highest in K. Neelkanth (6.9) followed by K. Ganga (6.4) and lowest in K Lima (2.9). Similar trend was recorded with regard to SMR by weight wherein highest SMR by weight was recorded in K. Neelkanth (25.1) followed by K. Ganga (20.3), but lowest was recorded in K. Garima (3.7) (Table 1). Non significant variation was reported in tuber number but significantly

higher tuber weight was recorded in Kufri Lauvkar (30.49 t/ha) over K. Mohan (26.59 t/ha) and K. Chipsona-1 (25.06 t/ha) under soil solarization study (Sadawarti *et al*, 2021). Similar variation for growth and yield parameters were reported among 6 varieties studied under successive double cropping of potato minitubers under insect proof net house conditions of N-W India (Kaur *et al*, 2021). Variability in terms of mean tubers weight and seed multiplication rate was reported in microplant, microtuber and recycled minituber of varieties in (Sadawarti *et al*, 2018) and microtuber multiplication study (Somani and Venkatasalam, 2012) confirming variation due to genetic makeup of the cultivar. In the present study, under 5 % samples with ELISA test health status were evaluated and were found sound.

The study demonstrated that there is significant variability among potato varieties in terms of emergence, yield attributes and Kufri Neelkanth emerged as the top-performing variety in the study, particularly in terms of yield and yield parameters.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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# EVALUATION OF POTATO BASED CROPPING SEQUENCE THROUGH ON-FARM DEMONSTRATIONS IN BUXAR, BIHAR

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**ABSTRACT:** With changing climate, diversification can be an alternative option for farmers to fight climate change related problems. Rice-wheat being the major cropping system followed in Indo-Gangetic plains need to be diversified with other crops to get higher productivity and additional income. Potato is an important cash crop for farmers of eastern plains during rabi season. In this context, current study was conducted in Buxar district of Bihar to evaluate the inclusion of potato in the existing sequence and find the yield gap at farmers' fields using on-farm demonstrations. Data was recorded through timely observation and was analyzed using descriptive statistics. The yield gap in potato in study area was found to be 26.4 per cent which can be bridged by using high yielding variety like *Kufri Mohan* and following the recommended package of practices. The economic analysis showed that farmers can earn nearly 40 percent more net return by adopting new varieties and scientific cultivation practices. Among five crop sequences evaluated at farmers' field, rice-potato-moong gave highest system productivity of 234 quintal per hectare as well as maximum net returns. Thus, it is advocated to include potato in the current cropping system which will boost productivity and income of farmers by manifold.

**KEYWORDS:** Cropping sequence, Bihar, demonstration, economics, system productivity

## INTRODUCTION

India is an agricultural country with smallholders dominating the overall landholding in this sector. Out of the total holdings, 89.4 per cent are in marginal and small farm categories having less than 2.0 ha land (PIB, 2023). Almost 55 per cent of the total workforce is employed in agricultural and allied sectors which have a share of 17.1 per cent in country's Gross Value Added (GVA) for the year 2017-18 at current prices (DAC & FW, 2019). The average monthly income per household in India stands at Rs 10218/-. Jharkhand (Rs 4895), Odisha (Rs 5112), West Bengal (Rs 6762) and Bihar (Rs 7542) are among the poorest states in terms of agricultural income/household (MoSPI, 2021). Changing climate and global warming are harsh realities faced by farming communities worldwide. Agriculture being the most climate

vulnerable sectors of Indian economy is likely to experience significant yield reduction in major crops. Due to climate change, irrigated wheat and maize yields may decline by 5-10 per cent by 2050 (ICAR Vision 2050). Rice yields will go down by 15-17 per cent in Punjab and Haryana and by 6-18 per cent in all other regions (CRIDA, 2013). Similarly, changing climate will also affect potato production in India. As per study, climate change may likely to benefit potato in Punjab, Haryana and western and central Uttar Pradesh by 3.46 to 7.11% increase in production, but in West Bengal and southern plateau region, potato production may likely to decline by 4 - 16% by 2030 (Reddy *et al.*, 2019). There is a need to enhance the productivity and profitability of farmers and reduce their vulnerability due to climate change by introducing innovative agricultural technologies.

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Rice-wheat cropping system is the most popular system followed across the eastern Indo-Gangetic plains area. Bihar is the third most populous state of India located in eastern region with a total population of 10.4 crore and area of 93.6 lakh hectares. Around 74 percent of the workforce in Bihar depends on the agricultural and allied sectors for their livelihood contributing almost 20 percent of the state's GSDP (Hoda *et al*, 2017). The small and marginal farmers have limited access to technology, inputs, credit, capital and markets. In Bihar, they frequently face problems of floods, drought, erratic rainfall etc. More than 90 per cent of farmers in Bihar are from small and marginal category and they grow paddy during *kharif* season and wheat during *rabi* season. These farmers cultivate wheat and rice using old varieties and traditional production practices. Potato is the most important vegetable crop of India. It is very nutritious food and has higher yield per unit area and time as compared to food grains like rice and wheat. Production of this crop is very effective means of converting plant, land, water and labour into a palatable and nutritious food (Sahadevan, 2007). India is the second largest potato producer in the world after China and both these countries together contribute nearly one third of total world potato production (Scott and Suarez, 2012). During the year 2020-21, India recorded a total of 56.17 million t of potato production from 2.20 million ha area with an average yield of 25.5 t/ha. Uttar Pradesh ranked first in terms of potato production followed by West Bengal, Bihar, Gujarat and Madhya Pradesh (Agricultural Statistics at a Glance, 2022). Bihar contributes around 15 per cent of total potato production. During the year 2020-21, Bihar recorded 90.41 lakh tonnes of potato production from an area of 3.27 lakh ha. As per forecast, India will require nearly 125 million t of potato from

an enhanced area of 3.62 million ha with an average yield of 34.5 t/ha during the year 2050 (CPRI, 2015).

Inclusion of potato in the prevalent cropping system can help in achieving this challenging task. Demonstration of high yielding potato varieties can increase the adoption rate of such varieties among farming communities. Keeping in mind the importance of potato in agricultural economy of Bihar, current study was undertaken to evaluate the demonstration of potato variety *Kufri Mohan* and potato based cropping sequence in Buxar district of Bihar.

## MATERIALS AND METHODS

### Selection of study area and sampling

The Climate Resilient Agriculture Program of Government of Bihar is being implemented by ICAR Research Complex for Eastern Region, Patna in two selected districts, *i.e* Buxar and Gaya. Potato was identified as one of the potential *rabi* crops for diversification of the existing cropping system in Buxar district alongwith chickpea, mustard etc. Therefore, Buxar district was selected purposively for this study. On farm demonstrations were conducted at farmers' fields in five selected villages of Buxar district of Bihar during *rabi* season for diversifying the existing rice-wheat cropping system and augmenting agricultural income. These villages namely Dalsagar, Balapur, Churamanpur, Harikishunpur and Ramobariya were selected in a cluster for the better impact of technology (Fig. 1). A total of 10 on farm demonstrations of high yielding new potato variety *Kufri Mohan* were carried out. *Kufri Mohan* is a variety having white-cream ovoid tubers with high yield (35-40 t/ha), moderate level of late blight resistance and good keeping quality. It was developed and released in 2016 for western

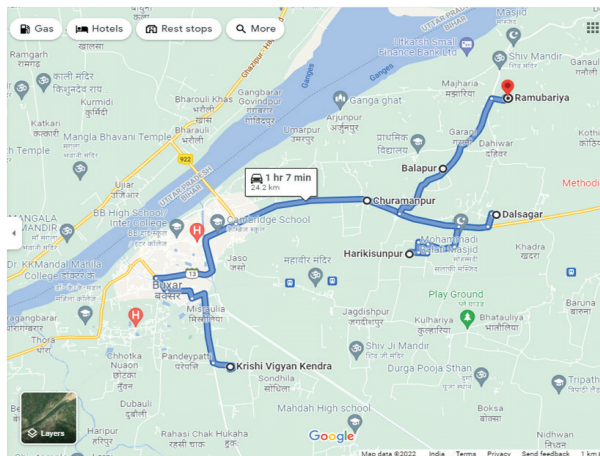


Fig.1. Map of Buxar showing cluster of selected villages

and eastern Indo-Gangetic plains by ICAR-Central Potato Research Institute, Shimla (Luthra *et al.*, 2017).

### Data collection and analysis

A participatory research design was used in this study. Farmers' Participatory Research (FPR) is an approach, which involves encouraging farmers to engage in experiments in their own fields so that they can learn, adopt new technologies and spread them to other farmers (Paris, 2023). Personal observation and survey method of data collection were employed for collection of primary data on variables like yield of potato, cost of cultivation, price etc. Secondary data were also collected from Government websites like Ministry of agriculture, Ministry of statistics and programme implementation, Press information bureau etc and published literature on area, production and yield of potato in India as well as Bihar. Technology interventions were made in the form of varietal demonstrations of the potato variety *Kufri Mohan*. Observations were recorded on yield, cost, price etc. The total yield gap (Yg) is the quantitative differences between a base-line yield (average farmers' yield) and either attainable (taken as experiment-based yield)

or potential yield ( $Y_p$ ) over some specified spatial and temporal scale (Sadras *et al.*, 2015). In this study, the total yield gap comprised of yield gap I and yield gap II. Yield Gap I (YG-I) is the difference between the potential yield ( $Y_p$ ) and yield of demonstration plot ( $Y_d$ ).

$$YG-I = Y_p - Y_d$$

Yield Gap II (YG-II) is the difference between the demonstration plot yield ( $Y_d$ ) and the actual yield at farmers' field ( $Y_a$ )

$$YG-II = Y_d - Y_a$$

Total yield gap was estimated using following formula:

$$\text{Total yield gap (TYG)} = Y_p - Y_a$$

Where,  $Y_p$  is potential yield of variety and  $Y_a$  is the actual yield at farmers' field.

Different cropping sequences including potato based sequence at farmers' fields were also evaluated in terms of system productivity and net returns. In this study, four cropping sequences i.e., Rice-Wheat-Moong, Rice-Potato-Moong, Rice-Lentil-Moong and Rice-Mustard-Moong were tried by providing quality seed of these crops and technical support. For estimating system productivity, rice equivalent yield of other crops was calculated by multiplying the yield of respective crops with its price and divided by price of rice. Collected data were tabulated and analyzed using descriptive statistics *viz.* mean, frequency, percentage etc. Inferences were drawn based on results and personal observations.

## RESULTS AND DISCUSSION

### Assessment of yield gap in potato

*Kufri Mohan* is a medium maturing, main season, high yielding table purpose potato variety suitable for cultivation in Indo-Gangetic plains. It has white creamy ovoid tubers with shallow eyes and white flesh. It has good keeping quality and moderate

dry matter (15-18%). The variety yielded 35-40 t/h (Luthra *et al.*, 2017). Potato in demonstration plot was grown using raised bed planting method with this high yielding variety *Kufri Mohan*. It was compared with the productivity of potato grown using traditional method using farmer's practice and shown in Table 1.

The results revealed that there was a significant difference in potato yield at on-farm demonstration plots and under farmers' practice. The yield level of *Kufri Mohan* realized on demonstration plots was 28.16 t/ha which was nearly 7 t/ha lesser than the potential yield. Thus, a yield gap of 19.5% was observed between potential yield at demonstration yield. The actual average yield under farmers' practice was even lesser at 25.8 t/ha. It is inferred that yield gap II which is the difference between the demonstration plot yield ( $Yp$ ) and actual yield ( $Ya$ ) was found to be only 8.5 per cent. Therefore, total yield gap (TYG) observed at Buxar was found to be 26.37% per cent at overall level. The findings of this study underscore the substantial impact of on-farm demonstration plots on potato yield compared to traditional farmers' practices. Higher yield of potato at demonstration plot could be attributed to improved potato variety used in demonstration plots and the fact that production technologies are on scientific lines and carried out under the

**Table 1: Yield gap in potato at sampled farms in Buxar, Bihar**

Sl. No.	Particulars	Actual yield/ yield gap (t/ha)	Percentage yield gap
1.	Potential yield* ( $Yp$ )	35.00	-
2.	On farm demonstration yield** ( $Yd$ )	28.16	-
3.	Actual yield under Farmers practice ( $Ya$ )	25.77	-
4.	Yield gap I ( $Yp-Yd$ )	6.84	19.54
5.	Yield gap II ( $Yd-Ya$ )	2.39	8.5
6.	Total yield gap ( $Yp-Ya$ )	9.23	26.37

Note: \*Taken as lower level of potential yield of *Kufri Mohan* (35-40 t/ha)

\*\*Yield of *Kufri Mohan* in demonstration plot at Buxar

supervision of scientists while farmers do traditional farming.

### Comparison of Economics of potato cultivation under demonstration and farmers' practice

The yield level and value of output per hectare for the demonstration plots and under farmers' practice is presented in Table 2. The gross return was estimated for both conditions by multiplying yield with average price of potato tubers *i.e* Rs 12000/- per ton. Thus, overall value of output per hectare came to Rs 337920/ha in demonstration plot, which was nearly 10% higher than under farmers' practice. The net return for demonstration plot was Rs 222320/ha which was significantly higher (41.56%) than farmer's practice mainly due to higher cost of potato cultivation under farmers'

**Table 2: Comparative economics of potato under demo plot and farmers' practice**

Sl. No.	Economic Parameters	Farmers' practice	Demonstration plots	Absolute change
1.	Average yield (tonnes/ha)	25.77	28.16	2.39
2.	Average price (Rs/t)	12000	12000	0.00
3.	Gross returns (Rs/ha)	309240	337920	28680
4.	Cost of cultivation (Rs/ha)	152200	115600	-36600
5.	Net returns (Rs/ha)	157040	222320	65280
6.	Cost of production (Rs/t)	5906	4105	-1801
7.	Input output ratio	1: 2.03	1: 2.92	-

practice. The substantial cost reduction in potato cultivation in demonstration plots, attributed to mechanization, contributed to this economic disparity. Cost of potato production was estimated to be nearly Rs 4.0/kg in demonstration plots which was Rs 2.0/kg lower than Farmers' practice. The overall input output ratio was found to be 1:2.9 in demo plot while it was only 1:2 in farmers' practice. Thus, farmers can earn Rs 3 after investing Rs 1 in potato cultivation for variety *Kufri Mohan*.

### Evaluation of cropping sequences at farmers' field

Rice-wheat-fallow is the traditional cropping system followed by farmers in Buxar district. Interventions under the climate resilient agricultural programme included study of various crop sequences and identification of most suitable cropping sequences at farmers' field. Rice was common crop in *kharif* and moong was common in summer season. Different *rabi* crops like wheat, potato, lentil and mustard were used in the sequences and data were recorded regarding grain yield.

It can be observed from Table 3 that potato based cropping sequence, i.e., Rice-Potato-Moong had the highest system productivity of 234.3 q/ha among all the five cropping sequences. It was followed by Rice-Wheat-Moong and Rice-Lentil-Moong having nearly equal system productivity of around 140 q/

ha. Very high system productivity in potato based sequences is mainly because of higher yield of potato per unit area and time. Therefore, if farmers can diversify existing system with potato in *rabi*, they can get more productivity.

Moreover, when income from these sequences are compared, rice-potato-moong tops with highest gross as well as net returns (Fig. 2). A farmer using this sequence can earn nearly Rs 3.5 lakhs per ha. If we compare it with traditional cropping system of rice-wheat-fallow having only Rs 55,000/ha net return, there is a huge boost in the income level of farmers. Therefore, it is advocated that we must include a third crop in summer for intensification of rice-wheat cropping system. Vegetables like potato can give very high returns if included in cropping system by farmers. Therefore, it is essential to acknowledge the volatility in potato prices, which may impact the consistency of such high returns. Careful consideration and strategic planning are therefore recommended when integrating potatoes or other crops into existing cropping systems.

### CONCLUSION

Being the most important vegetable of India, potato can be utilized effectively for diversifying the existing rice-wheat cropping system. Demonstration of scientific technologies at farmers' fields is an important

Table 3: Yield, system productivity and net returns of different crop sequences

Sl. No.	Cropping sequences evaluated	Yield (q/ha)			System productivity in quintal per hectare (q/ha)	Gross return Rs/acre	Net return Rs/acre
		<i>Kharif</i>	<i>Rabi</i>	Summer			
1.	Rice-Wheat-fallow (Traditional)	39.875	32.075	-	71.55	128800	54840
2.	Rice-Wheat-Moong	49.55	43.25	12.8	140.275	273965	99060
3.	Rice-Potato- Moong	50.625	309.55	11.775	234.3	524892.5	354760
4.	Rice-Lentil- Moong	48.725	15.85	12.725	139.825	268630	193695
5.	Rice-Mustard- Moong	48.625	14.175	12.7	130.025	253647.5	180505

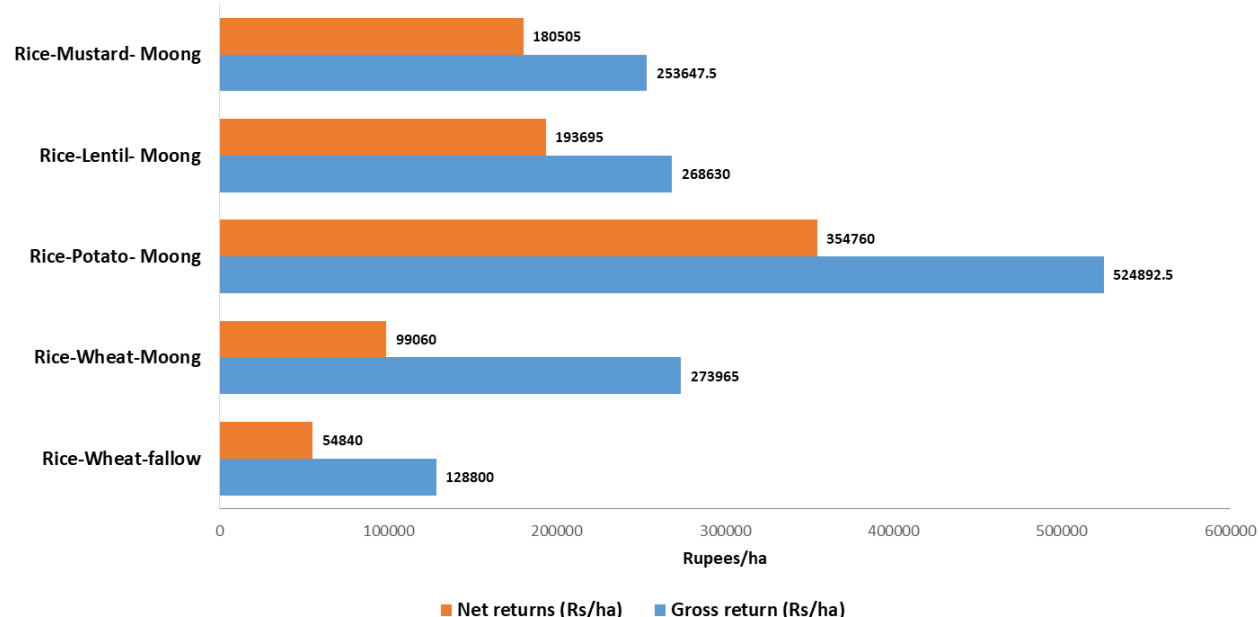


Fig. 2. Comparison of gross and net returns from various cropping sequences

method for adoption of these technologies by farmers. This study conducted in Buxar district of Bihar clearly showed that there exists a large gap in yield at farmers' field and potential yield of variety. Bridging this yield gap using suitable varieties of potato coupled with scientific management practices can enhance productivity of potato in the region. Moreover, farmers also get higher income by including potato in their existing cropping system. The present study has clearly shown the advantage of potato based cropping sequence over other sequences. Rice-wheat potato has been identified as most productive cropping sequence mainly due to higher yield of potato per unit area and time.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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### ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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# INFLUENCE OF ORGANIC AND INORGANIC FERTILIZERS ON GROWTH AND YIELD OF POTATO (*SOLANUM TUBEROSUM* L.) IN NYABIHU DISTRICT OF RWANDA

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**ABSTRACT:** The objective of this study was to evaluate the response of different organic and inorganic nutrients on physical growth and yield of potatoes in Nyabihu District. The experiments were conducted in farmer's fields in Nyabihu District during the agricultural seasons 2022 B (March - June 2022) and 2023 A (September 2022 – January 2023). The experiment was laid out in Randomized Complete Block Design (RCBD) with six treatments: T1 (Control), T2 (NPK), T3 (Compost), T4 (FYM), T5 (Compost + NPK), and T6 (FYM + NPK) in three replications. Physical growth and yield of the potato were evaluated. The collected data were subjected to ANOVA at 5 % level of significance and mean separation with Least Significant Difference (LSD). There was a significant difference in potato growth and yield at  $P < 0.05$ . Compost combined with NPK had the highest influence on physical growth and yield followed by farm yard manure combined with NPK in both seasons. The least was control preceded by NPK alone. Crops performed better in season 2023 A than 2022 B. Therefore, combination of organic and inorganic fertilizer synergically influence potato yield than when applied individually and it should be given special attention to increase potato yield.

**KEYWORDS:** Inorganic fertilizer, organic fertilizer, potato, yield increment

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most used vegetables. It belongs to the large family of vegetables Solanaceae (Burke, 2016; CIP, 2023). It is grown and eaten in many countries than any other crop and it is grown in all continents except Antarctica (Burke, 2016). It originated in the highlands of South America before Spanish explorers brought the plant to Europe in the late 16th century and thereafter spread in the entire planet (FAO, 2020). It ranks the third most important food crop in the world after rice and wheat in terms of human consumption (CIP, 2023). Rwanda is the first producer of

potato in East Africa and the third in Sub-Saharan Africa (USAID, 2016). More than a billion people worldwide eat potatoes, and global total crop production exceeds 374 million metric tons (CIP, 2023). Potato has gained an important consideration in human food due to its nutrients content.

Potato is essentially a food security crop with good prospects for increased domestic urban demand. It is mainly grown for source of food in many households and as a major source of income. It is among priority crops in Rwanda. Since its introduction, the Rwandan government has given it a substantial importance. Its production

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increased dramatically from 2,24,000 tons in 2013 to 6,000,000 tons in 2019 (Agriterra, 2020). Increase in potato production is linked to the increase in land utilized in potato production (Agriterra, 2020). Moreover, farmers supported by Agriterra noticed drastic increase in production per unit area from 10 t/ha in 2017 to 30 t/ha in 2020 (Agriterra, 2020). However, it was reported that average of productivity is still low being 8.6 t/ha in season A and 6.2 t/ha in season B of 2022 (NISR, 2022). This low productivity is related to different constraints in this sector which need to be dealt with in order to increase productivity of potato in all farmers.

Potato production in Rwanda is dominated by the use of inorganic fertilizers. During its introduction, mineral fertilizer was advocated to increase soil fertility and crop yield. It releases nutrients more rapidly which leads to the rapid development of the plant (Sharma and Chetani, 2017). However, in recent years mineral fertilizer was reported to increase soil acidity and cause nutrients imbalance (Atanaw and Zewide, 2021). Inorganic fertilizer has been reported that it can leach in soil, pollute water streams, water ponds and other water bodies (Sharma and Chetani, 2017). It can also leach away from the root zone, accumulate into the plant and harm people during consumption of food it contains (Sharma and Chetani, 2017). Accumulation of inorganic fertilizer was linked to high risk of cancer in humans (Sharma and Chetani, 2017). Consistent application of inorganic fertilizer leads to decline in soil micronutrients, depletion of organic matter, increase in acidity, degradation of physical property of soil and increase in erosion (Sharma and Chetani, 2017). Excessive application of nitrogen was linked to the reduction in quality of potato, environmental pollution, jeopardize public health, and leads to economic loss (Atanaw

and Zewide, 2021). Nowadays consumers and environmentalists are reluctant on the use of inorganic fertilizers and there is a need to seek for alternative of reducing inorganic fertilizer.

Inorganic fertilizer can be reduced by shifting to the organic fertilizer. Organic fertilizers derived from organic sources and are used to enrich soil nutrients in food production. Both chemical and organic fertilizers were reported to contribute morphological, physiological and biochemical performance of the crop (Sharma and Chetani, 2017). The use of organic fertilizer in soil amendment was reported to be environmentally friendly (Sharma and Chetani, 2017). Organic fertilizer prevents moisture loss, maintains soil temperature, facilitates microbial growth, prevents soil erosion and compaction; thus, maintaining soil fertility (Atanaw and Zewide, 2021). Organic fertilizer can be recommended for its healthy soil and moisture conservation potentiality. The benefits of organic fertilizers are enormous and this study aims at investigating its influence in potato production.

## **MATERIALS AND METHODS**

### **Experimental site**

The experiments were conducted in farmer's fields in Nyabihu District, Gisesero Cell during the agricultural seasons 2022 B and 2023 A. Nyabihu District is located in the western province of Rwanda. It is located on a volcanic mountain range whose altitude varies between 1800 and 2450 meters above sea level, 1°29' of Latitude S and 29°39' of Longitude E. The relief in Nyabihu is characterized by 90% rugged mountains with slopes of more than 55% creating a high risk of erosion. Soils comprise sandy and clay, laterite, and volcanic. Precipitation is almost uniform over every month and close

to 1400 mm per year. It has a climate with an average temperature of 15°C (Nyabihu District, 2019).

### Experimental design and treatments

Experiment was laid out in Randomized Complete Block Design (RCBD) in two agricultural seasons 2022 B and 2023 A with six treatments replicated three times. The elementary plots had a dimension of 4 × 3 m, spaced 1m in the blocks and 2 m between the blocks. The sowing density was 80 × 30 cm between and within rows, respectively, which gave a total of 50 planting holes per elementary plot, and 41,667 plants/ha. The potato seeds of the Kinigi variety were obtained from a certified seed multiplier in Musanze District. The first and second plowings took place from January through February of 2022 B and from August through September of 2023 A, and planting was undertaken at the beginning of the rainy season when there was enough rainfall for planting (Mid-March 2022 B, and Mid-September 2023 A seasons).

Treatment rates were:

- T1: Control (untreated),
- T2: (Farmer practice, 150 kg of NPK/ha),
- T3: (Compost, 3,000 kg/ha),
- T4: (Farm yard manure, 3,000 kg/ha),
- T5 : [(Compost (3,000 kg/ha) + NPK (150 kg/ha)],
- T6: [Farm yard manure (3,000 kg/ha) + NPK (150 kg/ha)].

### Data collection

In all seasons, randomly 5 plants were selected from each plot excluding border plants, and were used to measure various growth and yield parameters. The data on vegetative growth (plant biomass (number), plant height (cm), and plant diameter (cm)

were collected at the period interval of 15 Days After Planting (DAP) starting at 45 DAP to 105 DAP. Yield data was taken after harvesting. Technical data collection sheets, rulers, and weighing scales were availed for that purpose. All data on the physical growth and yield were collected at the same sampled plant from all plots.

### Statistical data analysis

Data were subjected to analysis of variance (ANOVA) appropriate to the RCBD in Microsoft Excel, and the treatment means were separated into homogeneous groups using the Least Significant Difference (LSD) at 5 % levels of significance.

## RESULTS AND DISCUSSION

### Plant leaf development (number)

Number of leaves of different treatments was significantly different at  $P < 0.05$  as presented in Tables 1 and 2. The number of leaves increased gradually from 45 DAP to 75 DAP in all sites and reduced at 90 DAP. In both seasons compost with NPK had the highest number of leaves and control was the lowest. Season 2022 B had the highest number of leaves than season 2023 A. Ratna *et al.* (2016) reported that the number of leaves was higher where NPK and compost were used together which is aligned with the results of this study. It was followed by the use of NPK alone while the control where no fertilizer was used was the least preceded by the use of combination of cow dung and poultry manure (Ratna *et al.*, 2016). Similarly, leaf index area followed the same trend of number of leaves (Ratna *et al.*, 2016). Organic manures were reported to increase the number of leaves (Suh *et al.*, 2015). The increase in number of leaves was also reported by Mama *et al.* (2016) due to combination of farm yard manure and nitrogen. Combination of organic manure

**Table 1: Plant leaf development from 45 DAP to 90 DAP in season 2022-B**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	41.1 <sup>bc</sup>	41.8 <sup>f</sup>	108.2 <sup>a</sup>	55.9 <sup>d</sup>
Compost + NPK	47.5 <sup>a</sup>	55.2 <sup>e</sup>	116.7 <sup>a</sup>	62 <sup>cd</sup>
Control	23.9 <sup>f</sup>	69.8 <sup>d</sup>	86.4 <sup>d</sup>	68.3 <sup>bc</sup>
FYM	34.3 <sup>d<sup>e</sup></sup>	75.8 <sup>c</sup>	92.5 <sup>cd</sup>	77.7 <sup>a</sup>
FYM + NPK	38.1 <sup>c</sup>	78.8 <sup>b</sup>	111.7 <sup>a</sup>	81.2 <sup>a</sup>
NPK	32.3 <sup>e</sup>	84.8 <sup>a</sup>	98.8 <sup>bc</sup>	86.2 <sup>a</sup>
General Mean	36.1	67.7	102.3	71.8
CV (%)	6.3	1.9	6.6	9.4
LSD (5%)	3.01	1.74	8.95	8.95

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5 % level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

**Table 2: Plant leaf development from 45 DAP to 90 DAP in season 2023-A**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	38.9 <sup>bc</sup>	71.9 <sup>c</sup>	92.2 <sup>a</sup>	74.2 <sup>a</sup>
Compost + NPK	45.3 <sup>a</sup>	80.9 <sup>a</sup>	100.7 <sup>a</sup>	82.7 <sup>a</sup>
Control	21.7 <sup>f</sup>	37.9 <sup>f</sup>	70.4 <sup>d</sup>	52.4 <sup>d</sup>
FYM	32.1 <sup>d<sup>e</sup></sup>	65.9 <sup>d</sup>	76.5 <sup>cd</sup>	58.5 <sup>cd</sup>
FYM + NPK	35.9 <sup>c</sup>	74.9 <sup>b</sup>	95.7 <sup>a</sup>	77.7 <sup>a</sup>
NPK	30.1 <sup>e</sup>	51.3 <sup>e</sup>	82.8 <sup>bc</sup>	64.8 <sup>bc</sup>
General Mean	34.0	63.8	86.3	68.3
CV (%)	6.71	2.07	7.85	9.92
LSD (5%)	3.01	1.74	8.95	8.95

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5 % level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting.

and NPK was reported to influence cell division, cell growth and proliferation of auxiliary branches (Atanaw and Zewide, 2021). Number of leaves increases gradually as the crop grows and it is influenced by the applied fertilizer. Combination of NPK and compost dominated other treatments in increasing number of leaves.

### Plant height (cm)

Plant height of different treatments was significantly different at  $P<0.05$  as depicted in Tables 3 and 4. Plant height increased gradually from 45 DAP to 90 DAP and reduced afterwards. Compost with NPK had the highest height and the least was for

the control. Season 2022 B had the highest plant height than season 2023 A. The effect of organic and inorganic fertilizer on plant height was also reported by Ratna *et al.* (2016) where combination of poultry manure and NPK resulted in the tallest plants 30 DAP to 75 DAP. Similarly, the least was for the control where no fertilizer was used preceded by the use of organic fertilizer alone (Ratna *et al.*, 2016). Moreover, plant height was also reported to be influenced by integration of cow dung and NPK compared to the control (Suh *et al.*, 2015). A combination of farm yard manure with NPK was reported to have a better increase in plant height than farm yard or NPK applied individually (Mama *et*

**Table 3: Plant height (cm) from 45 to 90 DAP in season 2022-B**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	47.5 <sup>a</sup>	87.7 <sup>a</sup>	101.2 <sup>a</sup>	102.2 <sup>a</sup>
Compost + NPK	48.1 <sup>a</sup>	96.2 <sup>a</sup>	109.7 <sup>a</sup>	110.7 <sup>a</sup>
Control	37.1 <sup>d</sup>	65.9 <sup>d</sup>	79.4 <sup>d</sup>	80.3 <sup>d</sup>
FYM	44.3 <sup>b</sup>	72 <sup>cd</sup>	85.5 <sup>cd</sup>	86.5 <sup>cd</sup>
FYM + NPK	47.3 <sup>a</sup>	91.2 <sup>a</sup>	104.7 <sup>a</sup>	105.7 <sup>a</sup>
NPK	41 <sup>c</sup>	78.3 <sup>bc</sup>	91.8 <sup>bc</sup>	92.8 <sup>bc</sup>
General Mean	44.2	81.8	95.3	96.3
CV (%)	3.85	8.29	7.11	7.04
LSD (5%)	2.25	8.95	8.95	8.95

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

**Table 4: Plant height (cm) from 45 to 90 DAP in season 2023-A**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	43.6 <sup>a</sup>	80.2 <sup>a</sup>	89.23 <sup>a</sup>	96.01 <sup>a</sup>
Compost + NPK	44.1 <sup>a</sup>	88.7 <sup>a</sup>	97.7 <sup>a</sup>	104.4 <sup>a</sup>
Control	33.1 <sup>d</sup>	58.3 <sup>d</sup>	67.36 <sup>d</sup>	74.14 <sup>d</sup>
FYM	40.4 <sup>b</sup>	64.4 <sup>cd</sup>	73.49 <sup>cd</sup>	80.2 <sup>cd</sup>
FYM + NPK	43.4 <sup>a</sup>	83.7 <sup>a</sup>	92.7 <sup>a</sup>	99.4 <sup>a</sup>
NPK	37.1 <sup>c</sup>	70.8 <sup>bc</sup>	79.84 <sup>bc</sup>	86.6 <sup>bc</sup>
<b>General Mean</b>	<b>40.3</b>	<b>74.3</b>	<b>83.3</b>	<b>90.1</b>
CV (%)	4.23	9.12	8.14	7.52
LSD (5%)	2.25	8.95	8.95	8.95

Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

*al.*, 2016; Atanaw and Zewide, 2021). Organic manure and inorganic fertilizer synergically increase plant height and optimum ratios should be use for a better growth.

### Plant stem diameter (cm)

Plant stem diameter was significantly different in both seasons for all treatments at  $P < 0.05$  as shown in Tables 5 and 6. There was a steady increase in plant diameter from 45 DAP to 75 DAP for season 2022 B and reduced at 90 DAP while for the season 2023 A there was a steady increase in number of leaves up to 90 DAP. Initially, in season 2022 B compost and NPK had the highest diameter of 2.2 cm which reduced to 1.3 cm

at 90 DAP. On the contrary, control was the least initially with 1.0 cm at 45 DAP which increased to 2.3 cm at 90 DAP. Overall, the highest increase was for farm yard manure for 1.7 to 2.5 after 45 and 90 DAP respectively. For season 2023 A, compost with NPK was the best throughout and control was the least. The study conducted in clay soil of Cameroon showed no significant influence of organic, inorganic fertilizers nor their combinations on the stem diameter of potato (Suh *et al.*, 2015). On the other hand, Mama *et al.* (2016) evaluated effect of different rates of farm yard manure and nitrogen and it was reported that the combination of the two influenced positively the increase in diameter of potato

**Table 5: Plant stem diameter (cm) from 45 to 90 DAP in season 2022-B**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	1.8 <sup>c</sup>	2.3 <sup>c</sup>	2.3 <sup>c</sup>	2.1 <sup>c</sup>
Compost + NPK	2.2 <sup>a</sup>	1.5 <sup>f</sup>	1.5 <sup>f</sup>	1.3 <sup>f</sup>
Control	1.0 <sup>f</sup>	2.5 <sup>b</sup>	2.5 <sup>b</sup>	2.3 <sup>b</sup>
FYM	1.7 <sup>e</sup>	2.7 <sup>a</sup>	2.7 <sup>a</sup>	2.5 <sup>a</sup>
FYM + NPK	2.0 <sup>b</sup>	2.2 <sup>d</sup>	2.2 <sup>d</sup>	2 <sup>d</sup>
NPK	1.7 <sup>d</sup>	2.2 <sup>e</sup>	2.2 <sup>e</sup>	2 <sup>e</sup>
General Mean (GM)	1.7	2.2	2.2	2.0
CV (%)	3.9	3.0	3.0	3.3
LSD (5%)	0.09	0.09	0.09	0.09

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

**Table 6: Plant stem diameter (cm) from 45 to 90 DAP in season 2023-A**

Treatments	45 DAP	60 DAP	75 DAP	90 DAP
Compost	1.8 <sup>bcd</sup>	2.3 <sup>abcd</sup>	2.2 <sup>bcd</sup>	2.2 <sup>abcd</sup>
Compost + NPK	2.2 <sup>a</sup>	2.7 <sup>a</sup>	2.6 <sup>a</sup>	2.6 <sup>a</sup>
Control	1.0 <sup>e</sup>	1.5 <sup>e</sup>	1.4 <sup>e</sup>	1.4 <sup>e</sup>
FYM	1.7 <sup>d</sup>	2.2 <sup>d</sup>	2.1 <sup>d</sup>	2.1 <sup>d</sup>
FYM + NPK	2.1 <sup>a</sup>	2.5 <sup>a</sup>	2.5 <sup>a</sup>	2.4 <sup>a</sup>
NPK	1.7 <sup>cd</sup>	2.2 <sup>cd</sup>	2.1 <sup>c</sup>	2.1 <sup>cd</sup>
General Mean	1.75	2.25	2.15	2.13
CV (%)	3.9	3.10	3.0	3.2
LSD (5%)	0.28	0.28	0.28	0.28

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Days After Planting

stem. Increase in stem diameter of potato due to combination of organic and inorganic fertilizer was also reported by Atanaw and Zewide (2021) where combination gave better results than when they were applied separately. Organic fertilizer in combination with inorganic fertilizer have positive influence on stem diameter.

### Yield of potato (t/ha)

Yield is important to evaluate effect of different treatments. There was a significant difference in yield for both seasons in all treatments at  $P < 0.05$  as presented in Table 7. Compost with NPK was the highest with yield

of 61.83 t/ha and 69.33 t/ha in season 2022 B and 2023 A respectively. It was followed by compost with 55.28 t/ha and 62.78 t/ha for 2022 B and 2023 A respectively. The lowest in both seasons was control. Ratna *et al.* (2016) studied effect of organic and inorganic fertilizers on yield of potato and they reported that combination of poultry manure and NPK had the highest yield followed by NPK alone, while the lowest yield was obtained from combination of cow dung and poultry manure and the control. The effect of organic fertilizer on potato productivity was also reported by Suh *et al.* (2015) where combination of cow dung and foliar gave the highest productivity

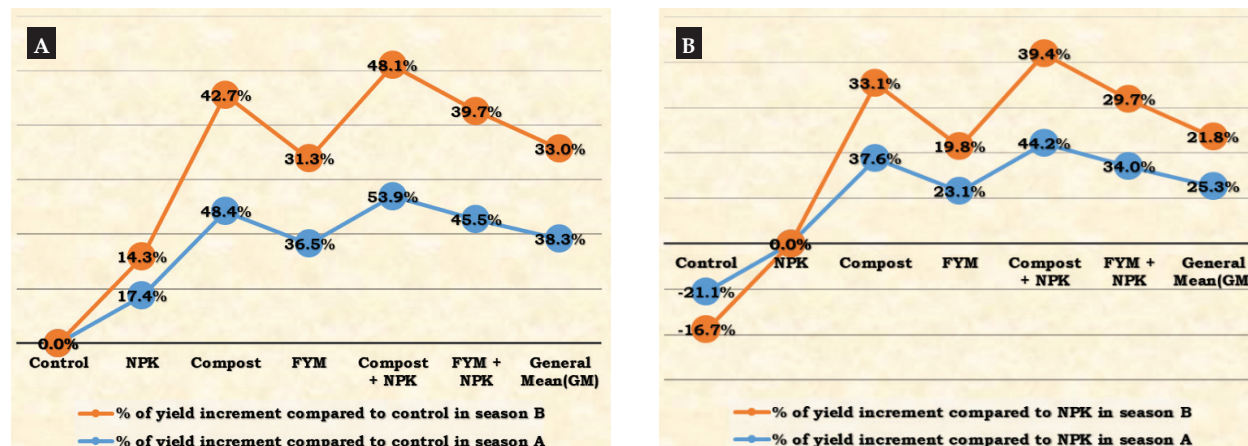


Fig.1. Percentage of Yield increment in seasons 2022 B and 2023 A compared to the control (A) and NPK (B)

Table 7. Comparative response of different organic and inorganic nutrient sources on the yield (t/ha) in the seasons 2022-B and 2023-A.

Treatment	Yield (t/ha) Season 2022-B	Yield (t/ha) Season 2023-A
Compost	55.28 <sup>bc</sup>	62.78 <sup>bc</sup>
Compost + NPK	61.83 <sup>a</sup>	69.33 <sup>a</sup>
Control	28.50 <sup>f</sup>	36.00 <sup>f</sup>
FYM	44.88 <sup>d</sup>	52.38 <sup>d</sup>
FYM + NPK	52.25 <sup>c</sup>	59.75 <sup>c</sup>
NPK	34.51 <sup>e</sup>	42.00 <sup>ef</sup>
General Mean (GM)	46.21	53.71
CV (%)	0.07	0.06
LSD (5%)	0.10	0.14

Means followed by the same letter(s) in a column are not significantly different by LSD at a 5% level of significance, CV=Coefficient of variation, LSD=Least Significant Differences, DAP=Day After Planting

in comparison to where inorganic fertilizer was used either alone or in combination with organic fertilizers. Similarly, control had the lowest yield compared to other treatments (Suh *et al.*, 2015). Therefore, potato yield can be maximized by combination of organic and inorganic fertilizers in specific ratios.

### Yield increment

Organic fertilizers influenced yield increment in both seasons as presented in figure 1. When compared to the control,

the average of yield increment was 33.0% in season 2022 B and 38.3% in season 2023 A. when control was taken as baseline, the highest increment was obtained when compost was used together with NPK and the lowest when NPK was used alone. On the other hand, when NPK was used as the baseline, the average of yield increment was 21.8% for season 2022 B and 25.3% for season 2023 A. Similarly, the highest increment was obtained when compost was used together with NPK, while control had (Atanaw and Zewide, 2021). Increment of potato yield due to combination of organic and inorganic fertilizer was also reported by other authors (Suh *et al.*, 2015; Ratna *et al.*, 2016). Combination of organic and inorganic fertilizer can be given much attention for effective increase in potato yield.

### CONCLUSION

The study on the response of different organic and inorganic nutrients on the physical growth and yield of potatoes (*Solanum tuberosum* L.) in Nyabihu District in agricultural seasons 2022 B and 2023 A revealed that organic fertilizers can increase significantly the physical growth and yield of potatoes. The combination of NPK with organic manure showed a better plant growth with a better yield. Compost with

NPK had the highest yield followed by farm yard manure with NPK in both seasons. Control had the lowest yield preceded by the application of NPK alone. Organic fertilizer when applied individually had a better yield than inorganic but still lower than combination of organic and inorganic fertilizers. Organic and inorganic fertilizers when applied together synergically increased potato yield. Therefore, to obtain a high yield, NPK should be applied with organic compost at specified ratios.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

### ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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## SEED POTATO GROWTH AND YIELD AFFECTED BY PLANT GROWTH REGULATORS UNDER SEED PLOT TECHNIQUE

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Potatoes, found as a dietary staple in numerous countries across the globe, inherently harbour a genetic capacity to produce substantial biomass within a limited timeframe per unit of land. In India, farmers have consistently cultivated this crop, even amidst significant price volatility and a scarcity of cold storage facilities. The commercial propagation of potatoes primarily relies on tubers, which serve as the pivotal foundation for projecting yields and concurrently constitute approximately 40% of the total cultivation expenses. This crop demands substantial inputs, particularly a significant quantity of seed tubers, for successful cultivation. The crop's overall performance hinges on the metabolic processes of the plant, especially during its growth and developmental stages. To address the challenge of achieving maximum production and productivity, plant growth regulators play a crucial role, even though they are needed in relatively small quantities. Research has shown that plant growth regulators can influence growth and contribute significantly to increasing yields by 10-15%, either by suppressing or stimulating plant growth (Birbal *et al.*, 2009). The growth and development of the seed potato are mainly due to the accumulation of

photosynthates and the response of available nutrients, which are regulated by the different hormonal balances in the plant system and can be modified by their external application. Out of the various plant growth regulators, gibberellins and auxins are primarily responsible for the growth and development of plants (Tekalign *et al.*, 2005). Rafeekher *et al.*, (2002) have shown that gibberellic acid is an important plant growth regulator that can be used to influence growth, yield and quality of potato. Singh *et al.*, (2005) studied the effects of gibberellic acid and 1-nepthalene acetic acid (NAA) on the growth and productivity of seed potato at Potato Research Station, Gwalior and observed increment in tuber yield by 18 percent with gibberellic acid application. The gibberellic acid gave a significantly higher number and weight of seed size (20-80g) tubers as compared to all other treatments. Alexopoulos *et al.*, (2006) observed the effect of gibberellic acid and its time of application on the growth of potato at Agricultural University of Athens, Greece and reported that the application of gibberellic acid increased the number of tubers per plant in potato. Therefore, this current investigation was initiated to explore the impact of foliar application of various plant growth regulators on the growth and yield of seed potato tubers.

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A field experiment was conducted for two consecutive years in randomized complete block design with cultivar *Kufri Pukhraj* at the Research Farm, School of Organic Farming, Punjab Agricultural University, Ludhiana, during the autumn seasons of 2019-20 and 2020-21. The experimental field's soil composition was characterized by a loamy sand texture, with a pH level within the normal range at 7.2 and electrical conductivity measuring 0.30 ds/m. Soil fertility analysis revealed that it possessed a medium organic carbon content of 0.50%, along with 288 kg/ha of available nitrogen. Additionally, the soil exhibited high levels of available phosphorus at 26 kg/ha and potassium at 335 kg/ha. The eleven growth regulation treatments were Control (Water spray), IBA [Indole-3-butyric acid] (100 ppm), IBA (200 ppm), NAA [1-Naphthaleneacetic acid] (25 ppm), NAA (50 ppm), Ethrel (25 ppm), Ethrel (50 ppm), GA<sub>3</sub> [Gibberellic acid] (100 ppm), GA<sub>3</sub> (200 ppm), Jeevamrit (Prepared by mixing 10 kg cow dung + 10 litres cow urine + 2 kg jaggery + 2 kg gram flour + handful of soil in 200 litres of water and fermented for 5-7 days) and Wastedecomposer (Prepared by mixing 2 kg jaggery + 1 bottle (30g) of waste decomposer in 200 litres of water and further diluting culture in the ratio of 1:3 with water). The treatments were applied at 45 and 60 days after sowing. Prior to field preparation, 50 tons per hectare of farmyard manure were applied, followed by pre-planting irrigation. The seed tubers of the *Kufri Pukhraj* variety were subjected to treatment with Monceren fungicide and allowed to sprout in a well-ventilated room at room temperature (20°C) for one week. These well-sprouted tubers were then planted in October, with a row spacing of 65 cm and a plant-to-plant spacing of 15 cm. The experimental layout featured plots measuring 3.25 m × 3.45 m, each containing 115 plants.

Immediately after planting, irrigation was administered to enhance emergence, followed by five additional irrigations up to the point of seed crop harvesting. The necessary nutrients, consisting of 187.5 kg of nitrogen, 62.5 kg of P<sub>2</sub>O<sub>5</sub>, and 62.5 kg of K<sub>2</sub>O per hectare, were supplied through urea, single superphosphate, and muriate of potash. At the time of sowing, half of the nitrogen dose and the full doses of phosphorus and potassium were applied, while the second nitrogen dose was top-dressed at 30 days after sowing (DAS). No insecticide was applied to the crop during both the years at both the locations (Ludhiana and Jalandhar). Plant height and leaf area index (LAI) were measured in each plot, with data collected from five randomly selected plants within each treatment during manual haulm cutting (86 DAS during 2019 and 87 DAS during 2020). Tuber and haulm weights were recorded using electronic balances from three randomly selected uprooted plants in each treatment during haulm cutting. Following the January harvest, the total yield of tubers and haulm for each net plot (6.72 m<sup>2</sup>) was weighed.

The plant height at 45 DAS did not vary significantly with different growth regulation treatments during both the years of study as the first application of growth regulators was made at this stage i.e., 45 DAS (Table 1). However, at 60 DAS, the significantly higher plant height than all the treatments were secured with the application of GA<sub>200</sub> (63.0 and 65.4 cm during the first and second year, respectively). Notably, the lowest plant height (35.3 and 39.5 cm during the first and second year, respectively) was acquired with Ethrel<sub>50</sub> than all the other treatments, except being statistically at par with Ethrel<sub>25</sub> and control during both the years. All the treatments except ethrel, jeevamrit and wastedecomposer had significantly higher plant height than the control. These findings

**Table 1: Effect of plant growth regulators on plant height and leaf area index**

Treatment*	Plant height				Leaf area index			
	45 DAS		60 DAS		45 DAS		60 DAS	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
Control	34.5	36.2	40.0	43.8	1.80	1.99	2.29	2.50
IBA <sub>100</sub>	34.3	37.0	53.1	55.6	1.79	1.96	3.03	3.18
IBA <sub>200</sub>	33.4	35.7	55.8	58.1	1.75	1.92	3.19	3.32
NAA <sub>25</sub>	33.2	37.3	46.6	48.3	1.73	1.98	2.66	2.76
NAA <sub>50</sub>	35.0	36.9	48.7	51.0	1.81	1.94	2.79	2.92
Ethrel <sub>25</sub>	34.7	37.6	38.1	42.4	1.78	1.96	2.18	2.42
Ethrel <sub>50</sub>	34.6	37.1	35.3	39.5	1.77	1.98	2.02	2.26
GA <sub>100</sub>	34.1	36.6	56.4	58.4	1.79	1.91	3.22	3.34
GA <sub>200</sub>	33.5	36.9	63.0	65.4	1.77	1.92	3.60	3.74
Jeevamrit	33.2	37.4	44.0	46.5	1.74	1.91	2.52	2.66
Wastedecomposer	33.3	36.8	42.8	46.4	1.76	1.97	2.44	2.65
LSD (p=0.05)	NS	NS	6.1	6.7	NS	NS	0.34	0.38

\*The subscripts in treatments are ppm values

closely align with those of Birbal *et al.*, (2009), who similarly asserted that gibberellic acid applied externally during the early tuberization phase improved plant height. The LAI at 45 DAS did not vary significantly with different growth regulation treatments during both the years (Table 1). However, at 60 DAS, the significantly highest LAI than all the treatments were obtained with application of GA<sub>200</sub> (3.60 and 3.74 during the first and second year, respectively). Significantly, lowest LAI (2.02 and 2.26 during the first and second year, respectively) was acquired with Ethrel<sub>50</sub> than all the other treatments, except being statistically at par with Ethrel<sub>25</sub> and control during both the years of study. Similarly, Mallick *et al.*, (2009) also reported an increment in LAI with growth regulation treatments.

The tuber weight per plant at 45 days after sowing (DAS) showed no significant variation among different growth regulator treatments in both study years (Table 2). However, at 60 DAS, the application of GA<sub>200</sub> resulted in significantly higher tuber weight

per plant compared to all other treatments, with values of 192.5 g and 201.1 g during the first and second years, respectively. Conversely, the lowest tuber weight per plant was significantly obtained with Ethrel<sub>50</sub>, measuring 132.2 g and 143.0 g during the first and second years, respectively. This weight was lower than all other treatments, except for Ethrel<sub>25</sub> and the control, which were statistically similar in both study years. These findings closely align with those of Alexopoulos *et al.*, (2007), who similarly noted a considerable increase in fresh potato weight with the administration of gibberellic acid when compared to the control and other growth regulators. The haulm weight at 45 DAS did not vary significantly with different growth regulation treatments during both the years of study. However, at 60 DAS, the significantly higher haulm weight per plant than all the other treatments were secured with the application of GA<sub>200</sub> (96.2 and 102.3 g during the first and second year, respectively). The lowest haulm weight per plant (58.4 and 64.4 g during the first and

**Table 2: Effect of plant growth regulators on tuber and haulm weight**

Treatment*	Tuber weight (g/plant)				Haulm weight (g/plant)			
	45 DAS		60 DAS		45 DAS		60 DAS	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
Control	47.8	48.9	142.8	152.9	51.7	55.0	64.2	69.5
IBA <sub>100</sub>	45.6	48.1	168.8	176.8	51.3	55.4	83.5	86.8
IBA <sub>200</sub>	48.0	50.3	176.6	184.3	51.4	56.9	86.7	92.2
NAA <sub>25</sub>	47.6	49.5	158.6	165.1	50.9	55.9	70.9	75.2
NAA <sub>50</sub>	48.3	51.1	162.6	170.4	52.4	54.9	76.0	80.1
Ethrel <sub>25</sub>	46.8	48.0	139.2	150.1	51.8	55.5	61.3	66.9
Ethrel <sub>50</sub>	46.7	49.9	132.2	143.0	51.9	55.9	58.4	64.4
GA <sub>100</sub>	47.7	49.9	175.7	183.1	50.9	54.8	84.8	90.8
GA <sub>200</sub>	47.8	52.3	192.5	201.1	51.5	56.5	96.2	102.3
Jeevamrit	46.2	49.5	153.9	161.7	51.5	56.5	70.0	74.6
Wastedecomposer	46.0	49.9	150.0	159.5	52.3	55.8	68.2	73.6
LSD (p=0.05)	NS	NS	14.6	16.0	NS	NS	8.5	9.2

\* The subscripts in treatments are ppm values

second year, respectively) was acquired with Ethrel<sub>50</sub> than all the other treatments, except being statistically at par with Ethrel<sub>25</sub> and control during 2019-20 and with Ethrel<sub>25</sub>, control and wastedecomposer during 2020-21. These results are in close conformity with El-Aal *et al.*, (2008), who also reported an increase in shoot weight with the foliar application of gibberellic acid in potatoes.

Total tuber yield differed significantly with different growth regulation treatments (Table 3). The significantly higher tuber yields (322.7 and 329.9 q ha<sup>-1</sup> during the first and second year, respectively) than all the other treatments were obtained with the application of GA<sub>200</sub>, which produced 34.9 and 31.5 percent higher tuber yields as compared to untreated control. Significantly lowest tuber yields (221.5 and 234.5 q ha<sup>-1</sup> during the first and second year, respectively) were observed with Ethrel<sub>50</sub> than all the other treatments except Ethrel<sub>25</sub>, control and wastedecomposer, which were statistically at par with Ethrel<sub>50</sub>. However, IBA<sub>200</sub>, GA<sub>100</sub> and IBA<sub>100</sub>, statistically at par with each other,

were significantly better than the control. The IBAs performed better than the NAAs among auxins. The Ethrel<sub>25</sub>, jeevamrit and wastedecomposer statistically at par with each other, had a non-significant influence on total tuber yield. All the growth regulation

**Table 3: Effect of plant growth regulators on tuber number and tuber yield**

Treatment*	Tuber yield (q/ha)	
	2019-20	2020-21
Control	239.2	250.9
IBA <sub>100</sub>	282.8	290.1
IBA <sub>200</sub>	295.9	302.4
NAA <sub>25</sub>	265.8	270.9
NAA <sub>50</sub>	272.4	279.5
Ethrel <sub>25</sub>	233.3	246.3
Ethrel <sub>50</sub>	221.5	234.5
GA <sub>100</sub>	294.5	300.3
GA <sub>200</sub>	322.7	329.9
Jeevamrit	257.9	265.3
Wastedecomposer	251.3	261.6
LSD (p=0.05)	24.4	25.8

\*The subscripts in treatments are ppm values

treatments except Ethrel<sub>50</sub>, Ethrel<sub>25</sub>, jeevamrit and wastedecomposer had significantly higher tuber yield than control. Similarly, Birbal *et al.*, (2009) reported the highest total tuber yield with the gibberellic acid application compared to other growth regulators.

The growth and yield attributes *viz.* plant height, leaf area index, tuber weight plant<sup>-1</sup> and haulm weight plant<sup>-1</sup> were significantly higher with foliar application of GA (200 ppm) than all the other treatments during both the years. The significantly lowest values of these parameters were secured with the foliar application of Ethrel<sub>50</sub>. Significantly higher total tuber yields were obtained with foliar application of gibberellic acid (200 ppm) at 45 and 60 days after sowing.

### CONFLICT OF INTEREST

The authors confirm that this manuscript has no conflict of interest

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Gram	g	Minute	min
Hectare	ha	Nanometre	nm
Kilogram	kg	Second	s
Litre	l	Square cm	cm <sup>2</sup>
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