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ORIGINAL ARTICLE

Variation in antioxidant status and productivity in andigena potato clones

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Abstract Potato can be a valuable source of antioxidants in the human diet, and genetic variability exists for antioxidant contents. The present study was carried out to evaluate the extent of variability in coloured flesh potatoes from andigena clones. There were significant differences among clones in ascorbic acid, phenols, total carotenoids and anthocyanins. During the two crop seasons, purple fleshed andigena potato clone JEX/A-122 was found to be superior in ascorbic acid, phenols and anthocyanins, followed by JEX/A-121. However, total carotenoids content in all the evaluated clones in general was less than half to that of yellow fleshed control variety Kufri Surya. Tuber dry matter in JEX/A-121 and JEX/A-122 was moderate (19.6 %) and statistically at par with that of Kufri Surya (21.1 %). Effect of growing season was also noticed on antioxidants status. The clones JEX/A-121 and JEX/A-122, superior in antioxidants showed significantly lower biological yield (226 and 246 g plant^{-1}) as compared to commercial varieties Kufri Surya and Kufri Pukhraj (661 and 665 g). Tuber yield in JEX/A-121(166 g plant^{-1}) and JEX/A-122 (156 g plant^{-1}) was also significantly lower than the variety Kufri Surya and Kufri Pukhraj (410 and 416 g plant^{-1}). The poor plant canopy and low yield in these clones was attributed to low leaf area, early senescence and reduced photosynthesis efficiency. Lower productivity in these clones negates the possibility of their direct exploitation towards either nutrient enhancement in potato driven food chains or as natural colourants. The genetic variability reported in this paper, however, provides impetus for future breeding work directed specially at enhancing the antioxidative matrix by targeted selections.

Keywords Antioxidant · Potato · Andigena clones · Tuber yield · Chlorophyll fluorescence

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Introduction

Antioxidants in plants have recently attracted great attention from the research community, food industry and consumers. A large number of scientific papers report the important role of phyto-chemicals in preventing many chronic diseases that are related to oxidative stress caused by free radicals (Vanderberg et al. 1999). Free radicals are associated with cancer inflammation, atherosclerosis and ageing. Phyto-chemicals such as polyphenols in fruits and vegetables possess high antioxidant activities that control degenerative oxidative reaction caused by reactive oxygen in living tissues (Kaur and Kapoor 2001; Lachman et al. 2005). Many studies have suggested beneficial effects in human health based on consumption of antioxidants as supplement or in the diet. Joseph et al. (2002) advocated the diets of highly pigmented foods, based primarily on the salutary effects of antioxidants and included red, purple and yellow-fleshed potatoes in the list of healthy foods.

The interest in functional foods has guided plant breeders of crops such as blueberries and potatoes (Prior et al. 1998; Reyes et al. 2004) to select genotypes with higher phenolic content, ascorbic acid etc. and antioxidant activity. Antioxidant capacity has been highly correlated to the amount of phenolic compounds and particularly anthocyanin present in dark coloured fruits and vegetables

(Prior et al. 1998; Brown et al. 2003). Potato is not ordinarily considered a food rich in antioxidants. However, the genetic variability in concentrations of anthocyanins, phenolic acids, ascorbic acid and carotenoids invites re-orientation of preconceptions (Brown 2005). Besides genetic constituents, several environmental factors affect potato yields and may influence the biosynthesis of antioxidants including anthocyanins and total phenols. On exposing to heat stress, higher levels of ascorbic acid, phenolic compounds and carotenoids were recorded in the tuber tissues in Kufri Surya (Kumar et al. 2007). Similarly, cool temperature and long days have been associated with increased growth and yields in potato (Vayda 1994), while location, light, and temperatures have been associated with red/radish anthocyanin biosynthesis (Giusti et al. 1998). The coloured potatoes have been shown to produce products such as snacks food high in antioxidants (Marwaha et al. 2008). Besides, the anthocyanin pigments of purple coloured potato may be of interest as natural food colourants. Hence, field evaluation of coloured andigena potato clones for antioxidants along with their growth and productivity traits would be an important step in determining their commercial value when grown in Indo-Gangetic plains. Andigena potato forms good parental material for breeding improved potato cultivars for Indian plains (Joseph and Gopal 1994) and their progeny shows heterosis for several economic characters (Tarn and Tai 1983). The overall objective of this paper was to analyse antioxidants, such as ascorbic acid, phenolic compounds, total carotenoids and total anthocyanins in freshly harvested and matured tubers of promising colored andigena potato [*Solanum tuberosum* ssp *andigena* (Juz. & Buk) Hawks] clones. Keeping this in view, six andigena clones previously assessed for coloured chips (Marwaha et al. 2008) were selected to enhance their potential breeding either as parental lines rich in antioxidants and/or targeted commercial use.

Materials and methods

A field experiment was conducted during the winter (*rabi*) seasons of 2010–2011 and 2011–2012 at the Central Potato Research Institute Campus Modipuram, Meerut (29°4′ N, 77°46′ E, 237 msl) in a randomized bock design with two replications. Treatments consisted of six andigena potato [*Solanum tuberosum* ssp. *andigena* (Juz. & Buk) Hawks] clones namely JEX/A-43, JEX/A-121, JEX/A-122, JEX/A-480, JEX/A-780, JEX/A-911 and two Indian varieties Kufri Pukhraj and Kufri Surya. The experiment was planted on 20 October in 2010–2011 and 2011–2012. Well sprouted seed tubers of 40–45 g seed weight were planted at a spacing of 60 cm × 20 cm in single row trial of 3.0 m. The plots were fertilized with 180, 80 and 100 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Half dose of nitrogen and full dose of P and K was applied at planting and remaining half dose of N was applied during earthing up at 25 days after planting (DAP). There was a minor rainfall (0.8 mm) during the crop season 2011–2012 (Table 1). Fungicides and insecticides were sprayed as and when required. Data recorded on growth and productivity variables for both the years showed similar trend and, therefore was pooled and analysed by computer software IRRISTAT, while the estimations of antioxidants were analysed on yearly basis and discussed accordingly.

Chlorophyll fluorescence

To assess the efficiency of photosynthetic light reaction, chlorophyll fluorescence was recorded at 60 and 90 DAP in the field with a continuous excitation portable Fluorometer (Handy Pea, Hansatech Instruments Ltd. King's Lynn, Norfolk Pe32 IJL England). It uses a 650 nm radiation to provide the saturating pulse and detects at wavelength above 730 nm. Measurements were made on leaflets of fully expanded leaves of the upper canopy after a 20 min dark adaptation period between 11:00 and 13:00 (local solar time). Dark adaptation time was the time needed to obtain a steady value of variable fluorescence (F_v) and maximum fluorescence (F_m). Leaf clips were applied to fully sun exposed leaflets of three potato plants randomly sampled in the centre of each row and measurements on F_v/F_m were made with saturation irradiance up to 3,000 μmol m^{-2} s^{-1}.

Growth and yield traits

Randomly, two plants per row were removed, washed and separated into haulms (leaves and stems), roots and tubers. Leaf area was estimated using table top Leaf Area Meter, model LI-3100 (LI-COR, Lincoln, NE USA) twice at 60 and 90 DAP, while fresh weight of different plant parts was recorded only at 90 DAP. Total biological yield and harvest index was calculated and are presented on per plant basis. Remaining plants were dehaulmed manually at 90 days and harvested 10–15 days later, once tuber skin was optimally hardened. Tuber shape, size, skin colour, flesh colour, depth of eyes were recorded (Table 2). Three medium size tubers were taken from each clone/variety and chopped into small pieces to estimate tuber dry matter content. A representative sample of 50 g was drawn and kept in forced hot air oven at 80 °C until constant weight was achieved.

Antioxidants status

Five tubers of uniform size were selected at random from each replication and genotype at harvest. These were

Table 1 Monthly value of meteorological parameters during crop seasons

Month	2010–2011					2011–2012				
	Mean air temperature (°C)			BSS[a] (h)	Rainfall (mm)	Mean air temperature (°C)			BSS[a] (h)	Rainfall (mm)
	Max	Min	Mean			Max	Min	Mean		
October	34.5	11.0	22.7	7.2	0.0	33.5	14.5	24.0	6.3	0.0
November	32.5	6.5	19.5	5.8	0.0	30.0	9.5	19.8	2.9	0.0
December	27.5	2.0	14.8	5.2	0.0	28.0	1.5	14.8	4.6	0.0
January	21.0	2.5	11.8	4.1	0.0	20.5	3.5	12.0	4.0	0.8

[a] Bright sunshine hours

Table 2 Morphological features of andigena potato breeding clones and varieties

S. no.	Clone/variety	Tuber shape	Mean tuber size (mm)	Skin colour	Flesh colour	Eyes
1.	JEX/A-43	Round	50	Light Brown	Yellow	Medium deep
2.	JEX/A-121	Round	34	Light purple	Light purple	Shallow
3.	JEX/A-122	Round	32	Dark purple	Dark purple	Medium deep
4.	JEX/A-480	Round	32	Purple	Light purple	Deep
5.	JEX/A-780	Round	33	Brown	Yellow	Deep
6.	JEX/A-911	Round	50	Purple	Light yellow	Medium deep
7.	Kufri Pukhraj	Ovoid	55	Yellow	Yellow	Deep
8.	Kufri Surya	Oblong	60	White cream	Pale yellow/cream	Shallow

washed, peeled, cut into small pieces and mixed thoroughly. A representative sample of tuber tissues was drawn and subjected to analysis for antioxidants as described below.

Ascorbic acid content was estimated by the method described by Bajaj and Kaur (1981). Five gram of composite tuber tissue was macerated in 100 ml of 4 % oxalic acid and filtered using Whatman No. 1 filter paper. To 5 ml of this supernatant, 10 ml of 4 % oxalic acid was added and titrated against the dye dichloro-phenol indo-phenol (DCPIP).

Total phenols content were estimated by the method described by Swain and Hillis (1959). Ten grams composite tuber sample was extracted in 80 % isopropyl alcohol for 3 h at 70 °C. The extract was pooled and the filtrate was evaporated to dryness and the volume made to 100 ml with distilled water. To 1.0 ml of filtrate, 0.5 ml of folin reagent was added for colour development. Standard curve was prepared using chlorogenic acid.

For total carotenoids content estimation 20 g tissue was macerated and extracted in 100 ml of cold acetone: *n*-hexane mixture (75:60 v/v) twice or till the filtrate was colourless. The filtrate was taken in a separating funnel and cold water was added. The contents were shaken slightly to make it free of acetone. The hexane layer containing carotenoids was dried over anhydrous Na_2SO_4 and volume was made to 100 ml with hexane. The absorbance was read at 436 nm in spectrophotometer. The total carotenoids content of tubers was calculated as described by Thomas and Joshi (1997) and expressed as µg carotene 100 g fr. wt.$^{-1}$.

Anthocyanin extraction followed the protocols of Thimmaiah (1999). Ten g of tissue was grinded in 50 ml of alcohol and methanolic HCL (1:1) in a conical screw cap tube using a vortex mixture. To the 4 ml filtrate, 2 ml of anthocyanin reagent was added and the reaction mixture was incubated in dark for 20 min against blank. The absorbance was measured at 525 nm with a UV VIS Double Beam spectrophotometer (model ELICO SL 210) using anthocyanin extraction solvent as the blank. The amount of anthocyanin present in the samples was calculated from standard curve prepared with cyanin hydrochloride.

Results and discussion

Antioxidants

During 2010–2011, clone JEX/A-122 was superior in ascorbic acid (34.4 mg 100 g^{-1} fr. wt.) and phenols (71.0 mg 100 g^{-1} fr. wt.) as compared to control variety Kufri Surya (24.4 and 57.1 mg, respectively) and mean of all genotypes (24.3 and 57.9 mg; Table 3). Total carotenoids in andigena clones was half [ranged from 20.5 µg to 40.3 µg (100 g)$^{-1}$ fr. wt. in JEX/

A-122 and JEX/A-43, respectively] to that of Kufri Surya (110 μg (100 g)$^{-1}$ fr. wt.). Antioxidant activity of potato can be affected by genotype and tuber flesh colour (Brown et al. 2003). Anthocyanin content was highest [2,250 μg (100 g)$^{-1}$ fr. wt.] in JEX/A-911. Since this clone has very dark purple colour skin, while flesh is yellow (Fig. 1), therefore, majority of anthocyanin content appeared to be confined in skin only. The next higher anthocyanin content (1,316 μg) was observed in JEX/A-122, with dark purple tuber flesh (Table 2), which suggests that bulk of the anthocyanin was present in its flesh tissues (Brown 2005). Purple and red coloured potato genotypes have been associated with the presence of flavonoids in the skin and flesh (Brown 2005) and anthocyanin were found to be concentrated more in purple coloured tissues. Considerable differences of the total polyphenols content between yellow and purple-fleshed potatoes have been reported by Lachman et al. (2008).

Anthocyanins are among the many flavonoids that are found in potato tubers. A series of single genes controls the presence and absence of red and blue pigments. Different genetic systems controlling pigment expression have been identified for diploid cultivated vs tetraploid cultivated potatoes (Lunden 1960). These estimations suggest that JEX/A-122 was superior in three antioxidants namely ascorbic acid, phenols and anthocyanins during 1st crop season (Table 3).

The pattern of antioxidant contents was slightly different during 2011–2012. Across the clones and varieties, the mean values of ascorbic acid and anthocyanin contents decreased significantly during 2011–2012, while phenols and carotenoids contents increased. Effect of growing season and location on antioxidants status was also been reported earlier by Vayda (1994) and Giusti et al. (1998). Besides, antioxidant activity of potato can be affected by genotype, tuber flesh colour, cooking method and spatial location within potato tubers (Brown et al. 2003). Crop season 2011–2012 also witnessed 1 °C higher temperature (difference between maximum and minimum) in the month of December (Table 1), which coincided with the active phase of tuber development. Variations in temperature regime are known to

Fig. 1 Skin colour of andigena clones and cultivated varieties of potato

Table 3 Antioxidant status of potato breeding clones and varieties

Clone/ variety	Ascorbic acid [mg (100 g)$^{-1}$ fr. wt.]	Phenols [mg (100 g)$^{-1}$ fr. wt.]	Carotenoids [µg (100 g)$^{-1}$ fr. wt.]	Anthocyanins [mg (100 g)$^{-1}$ fr. wt.]
2010–2011				
JEX/A-43	16.7	50.5	42.3	58.0
JEX/A-121	25.3	52.7	28.0	105.0
JEX/A-122	34.4	71.0	20.5	1316.0
JEX/A- 480	28.1	50.9	32.0	821.0
JEX/A-780	20.2	79.0	26.1	289.0
JEX/A-911	30.3	62.0	31.0	2250.0
Kufri Pukhraj	14.9	39.8	49.8	90.0
Kufri Surya	24.4	57.1	110.5	150.0
Mean	24.3	57.9	42.5	634.8
2011–2012				
JEX/A-43	17.9	46.5	48.6	300.0
JEX/A-121	29.0	100.0	72.8	695.0
JEX/A-122	27.5	98.3	48.3	915.0
JEX/A- 480	21.9	62.2	50.3	325.0
JEX/A-780	18.2	71.8	71.1	185.0
JEX/A-911	17.7	60.4	47.5	830.0
Kufri Pukhraj	11.3	46.6	39.5	94.0
Kufri Surya	20.2	68.8	80.5	265.0
Mean	20.4	69.3	57.3	451.1
LSD ($p \leq 0.05$)				
Year (Y)	0.5	1.7	1.4	36.6
Clone/variety (V)	1.0	3.4	2.9	73.2
Interaction Y × V	1.4	4.8	4.1	103.6

influence antioxidant contents in plant tissues (Vasquez-Robinet et al. 2008). Effect of location and probable role of heat and drought stresses have been reported by Hamouz et al. (2011), as significantly higher total anthocyanin content was found in a lowland Přerov nad Labem area of Czech Republic, which could be probably related to drought stress. Giusti et al. (1998) have also reported the effect of location, light, and temperatures with red radish anthocyanin biosynthesis. The ascorbic acid [29.0 mg (100 g)$^{-1}$ fr. wt.] and phenols [100 mg (100 g)$^{-1}$ fr. wt.] content were highest in JEX/A-121 as compared to Kufri Surya [20.2 and 68.8 mg (100 g)$^{-1}$ fr. wt.], and clone JEX/A-122, which had highest value in previous year performed at par with JEX/A-121 both in ascorbic acid [27.5 mg (100 g)$^{-1}$ fr. wt.] and phenols [98.3 mg (100 g)$^{-1}$ fr. wt.]. Besides, total carotenoids content in 2011–2012 increased in JEX/A-122 [48.3 µg (100 g)$^{-1}$ fr. wt.] over the value in previous year (20.5 µg), however values were still significantly lower than Kufri Surya [80.5 µg (100 g)$^{-1}$ fr. wt.]. As in previous year, the total anthocyanins were also highest in JEX/A-122 [915 µg (100 g)$^{-1}$ fr. wt.] as compared to mean of all the genotypes (451 µg). These results confirmed that clone JEX/A-122 was superior in ascorbic acid, phenols and anthocyanins during both the crop seasons and is likely to have greater nutritional value in terms of phytochemicals followed by JEX/A-121.

Growth and productivity

Data on growth and productivity traits indicated a generally low biological yield (BY) and poor plant canopy (haulm, root and tuber weight) in all the andigena clones (Table 4). The clones superior in antioxidants namely JEX/A-121 and JEX/A-122 had about 1/3rd and significantly lower BY (226 and 246 g) as compared to commercial varieties Kufri Surya and Kufri Pukhraj (661 and 665 g plant^{-1}). Tuber yield in JEX/A-121 (166 g plant^{-1}) and JEX/A-122 (156 g plant^{-1}) was also significantly lower than the variety Kufri Surya and Kufri Pukhraj (410 and 416 g plant^{-1}). Among the andigena clones, JEX/A-911 showed highest biological yield and tuber yield viz., 420 and 239 g, respectively, however, its performance in antioxidants contents was significantly less than JEX/A-121 and JEX/A-122. Singh et al. (2005) also recorded low tuber yield in hybrid JH-214, superior in antioxidants, than the standard varieties. The poor plant canopy and low yield in these clones could be ascribed to low leaf area and reduced photosynthetic efficiency.

Table 4 Growth and productivity traits at 90 DAP in potato clones and variety

Clone/ variety	Haulms fr. wt. (g plant^{-1})	Root fr. wt. (g plant^{-1})	Tuber fr. wt. (g plant^{-1})	Total biological yield (g plant^{-1})	Harvest index (%)	Tuber dry wt. (%)
JEX/A-43	58.8	4.7	87	151	58	17.1
JEX/A-121	54.9	5.3	166	226	73	19.6
JEX/A-122	74.1	15.7	156	246	63	19.6
JEX/A- 480	95.2	6.5	99	201	49	17.8
JEX/A-780	88.9	4.2	124	217	57	23.4
JEX/A-911	155.7	25.1	239	420	56	24.6
Kufri Pukhraj	220.1	28.6	416	665	63	15.4
Kufri Surya	220.2	30.4	410	661	62	21.1
Mean	121.0	15.1	212	348	60	19.8
LSD ($p \leq 0.05$)	23.6	7.7	71	94	7.4	0.9

Values are based on pooled data of 2010–2011 and 2011–2012

Perusal of leaf area development indicated that JEX/A-121 and JEX/A-122 had moderate (1,304 and 1,500 cm^2 plant^{-1}) and comparable values with standard varieties (1,222 and 1,276 cm^2 in Kufri Pukhraj and Kufri Surya, respectively) at 60 DAP, but thereafter it declined drastically in the clones, while continued to increase in the varieties up to 90 DAP, resulting into four times the leaf area compared to clones (Fig. 2). This pattern of leaf area growth suggest that the leaf senescence was set-in early and faster, particularly in andigena clones JEX/A-43, JEX/A-121 and JEX/A-122, as compared to moderate yielder clone JEX/A-911 and control variety Kufri Surya and Kufri Pukhraj. Leaf area was lowest in clone JEX/A-480 (573 cm^2), followed by JEX/A-780 (984 cm^2) at 60 DAP and it remained considerably lower than the control varieties at 90 DAP, which led to very low tuber yield in these clones. Clone JEX/A-911 showed excellent leaf growth both at 60 and 90 DAP viz., 2,055 and 1,827 cm^2, respectively, which helped in achieving good productivity in this clone.

F_v/F_m as proposed by Butler and Kitajima (1975) is a useful parameter, which is proportional to the quantum yield of photosystem 2 (PS II) photochemistry and exhibits a high correlation with the quantum yield of net photosynthesis. Averaged over seasons, JEX/A-121 and JEX/A-122 had lower values of F_v/F_m, viz., 0.42 and 0.51 at 60 DAP and 0.40 and 0.40 at 90 DAP, respectively, indicating that these two clones had lower efficiency of photochemical process in PS II and quantum yield of net photosynthesis than the other clones (Fig. 3). Lower capacity (reduced leaf area at 90 DAP) and lower efficiency (low F_v/F_m) may be associated with lower productivity in JEX/A-121 and JEX/A-122, suggesting a poor possibility for exploiting them through commercial cultivation. F_v/F_m was found as one of the most reliable chlorophyll fluorescence parameters for the estimation of genotypic differences, and

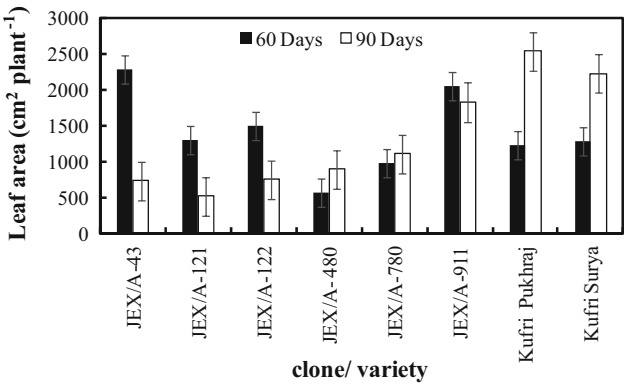

Fig. 2 Leaf area of andigena clones and cultivated varieties of potato

could represent a practical means to discriminate between genotypes under field condition (Ierna 2007). As in case of leaf area, the F_v/F_m ratio was also higher in JEX/A 911 both at 60 and 90 DAP, 0.72 and 0.70, respectively, which was comparable to commercial varieties and contributed in achieving highest biological yield and tuber yield among the clones evaluated in this study. Presence of high dry matter is a preferred quality trait both for table and processing potatoes. Tuber dry matter in JEX/A-121 and JEX/A-122 was moderate (19.6 %) and statistically at par with that of Kufri Surya (21.1 %). The highest TDM was recorded in JEX/A-911 (24.6 %), followed by JEX/A-780 (23.4 %), and lowest in Kufri Pukhraj (15.4 %). Results on dry matter contents in andigena clones are in agreement with earlier reports (Joseph and Gopal 1994; Marwaha et al. 2008).

Coloured potatoes could be an excellent source of antioxidant rich ingredient for the production of nutritionally enhanced food products. Present study indicated that ascorbic acid, total phenols, total anthocyanin were higher in two purple fleshed andigena potato clones namely JEX/

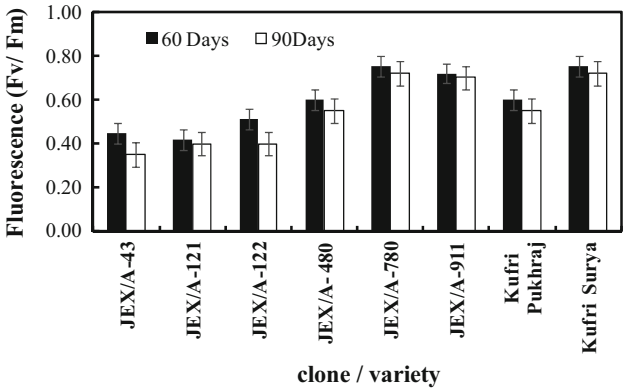

Fig. 3 Fluorescence characteristics of andigena clones and cultivated varieties of potato

A-121 and JEX/A-122 as compared with those of yellow/cream fleshed control varieties. Skin and flesh colour appeared to be directly associated with higher antioxidant contents. The genetic variability reported in this paper provides impetus for future breeding work directed specially at enhancing the antioxidative matrix by directed selection for higher concentrations of antioxidants. Colourful skin and flesh, especially purple in JEX/A-121 and JEX/A-122, tended to have higher antioxidant content than yellow coloured flesh. Li et al. (2006) opined that as the colour intensity has a strong correlation with antioxidants, these visible traits provide suitable selection criteria for high antioxidant varieties. Breeders may consider these clones in the breeding programme for increasing nutritional value of the potato crop. Lower productivity in clones with higher antioxidants, however, negates the possibility of their direct exploitation towards either nutrient enhancement in potato driven food chains or as natural colourants.

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