



# Evaluation of Dry Matter, Starch Content and $\beta$ -carotene in $F_1$ Progenies of a Cross between White-fleshed and Orange-fleshed Sweet Potato

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## Abstract

Sweet potato (*Ipomoea batatas* (L.) Lam.) is one of the valuable crops producing the highest root dry matter content for human consumption. Sweet potato breeding is very challenging due to its genetic complexity. The tuber flesh colour variation ranges from white, cream, yellow, orange and purple. High dry matter content, carotene and anthocyanins are the main tuber characters preferred by consumers and processors. In this study, a population consisting of 200  $F_1$  progenies from a cross of S-1 (white fleshed) and ST-14 (orange fleshed) were analyzed for dry matter, starch and  $\beta$ -carotene content. The dry matter content of the progenies ranged from 18.58% (OW48) to 45.7% (OW15) and 30 per cent of the progenies showed a dry matter content of 35 - 40%. The starch content accounted variations from 9.80 to 32.70 per cent and only 9 per cent showed starch content above 30 per cent. Among the progenies,  $\beta$ -carotene ranged from 0.02 – 11.03 mg/100g FW. The progenies OW4, OW27, OW37, OW43, OW126 and OW169 recorded the highest dry matter,  $\beta$ -carotene as well as high starch content. Among the progenies orange flesh colour was predominant and from the OFSP progenies, the most predominant tuber flesh colour observed was light orange (28%), orange (22%) and deep orange (4%) colour respectively.

**Key words:** Sweet potato, pollination, tuber flesh colour, dry matter, starch content,  $\beta$ -carotene

## Introduction

Sweet potato (*Ipomoea batatas* L. Lam) is a dicotyledonous tuber crop belonging to the morning glory family, Convolvulaceae. It is the world's seventh most important food crop, grown more in developing countries than any other tuber crops. The annual production of sweet potato is estimated to be 110.7 MT from an area of 8.2 M ha with a productivity of 13.5 t ha<sup>-1</sup>. China is the world's largest producer of around 79 MT from about 3.5 M ha. India comes ninth in production which is about 1.13 MT from an area of 0.11 M ha with a productivity of 10.2 t ha<sup>-1</sup>, of which maximum is contributed by Odisha state (FAO, 2013).

Besides its importance as human food, sweet potato provides raw material for industrial purpose and also used

as animal feed. The tuber flesh colour variation in sweet potato is significant and a noticeable variation includes white, cream, orange and purple depending upon the presence or absence of the pigment. Orange-fleshed sweet potato has much relevance in developing countries as it contains significant amounts of  $\beta$ -carotene, dietary fibre, minerals, vitamins which has the potential to prevent malnutrition and enhance food security of the country (Diaz et al., 1996). The composition and contents of nutrients in tuber vary greatly depending upon the genetic and environmental factors (Bovell-Benjamin, 2007). The consumption of carotenoid-rich foods is related to prevention of cancer, cardiovascular diseases and other degenerative processes involving oxidative stress (Willet, 1990; Sies and Stahl, 2003).

The sustainability and expansion of sweet potato production depends on the availability of varieties that meet end-users preferences and are possible only through sweet potato breeding. The seedlings developed from hybridization showed a marked variation in leaf shape, skin and flesh colour, tuber shape and other characteristics due to high heterozygosity. A number of true hybrids were developed and released for cultivation in different countries by combining the desirable characteristics of two or more selected parents. Incompatibility in sweet potato is the main barrier to the production of seeds (Martin, 1965; Martin and Cabanillas, 1966). Self-fertilisation in sweet potato is rare due to self-incompatibility and cross incompatibility.

The selected parents were S-1 (male parent) variety which is a spreading type with green colour vine and emerging leaf. Leaves are narrowly 5 lobed and tubers are elliptical in shape with purple skin and white flesh colour. The dry matter content of the tuber was 37 per cent and very sweet in taste. The colour of the flower and flesh is white with good latex content. The female parent ST-14, is a semi-erect type with mostly dark purple vine with green emerging leaf colour and is triangular in shape with purple flower. Tubers are ovate type with cream colour skin and dark orange flesh colour with dry matter of 27 percent and contain high  $\beta$ -carotene content.

Vimala and Binu Hariprakash (2011) had reported on the range of variability and the segregation pattern for the different morphological characters and the inheritance pattern of the carotenoids in the hybrid progenies developed from a cross between an orange-fleshed and white-fleshed sweet potato. Vimala et al., (2012) also evaluated for storage root yield,  $\beta$ -carotene and dry matter content within the selected 42 orange-fleshed sweet potato hybrids and to identify high yielding hybrids with high  $\beta$ -carotene and dry matter content.

The present study targeted to the development of  $F_1$  progenies from a cross of S-1 and ST-14 parents and the progenies were evaluated for their variability in dry matter, starch and  $\beta$ -carotene content in seedling population.

## Materials and Methods

### Selection of parents from elite clones

From the sweet potato germplasm, 85-100 clones with varying intensity in tuber flesh colour, including orange-

fleshed sweet potato (OFSP) and white-fleshed sweet potato (WFSP) with high dry matter content were selected for flowering study. Among the clones, 11 OFSP and 8 WFSP clones, which showed high  $\beta$ -carotene and dry matter contents were selected and evaluated in two seasons, viz., May – July 2011 and September - November 2011.

After the evaluation study the S-1 variety was selected as the male parent. The dry matter content of the tuber was 37 per cent and very sweet in taste. The female parent ST-14, is triangular in shape with purple flower. Tubers are ovate type with cream colour skin and dark orange tuber flesh with high  $\beta$ -carotene. Hence, the two varieties namely ST-14 (OFSP) and S-1 (WFSP) were selected for the breeding purpose. The pollination of the selected parents was done at the field of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, during 2013.

### Development of mapping population

#### Crossing block

Sweet potato flowers best during October to December with short day conditions. Therefore, winter is the best time for seed production. The vine cuttings of the two selected parents were collected and planted in the mounds in rows and columns. Each parent was planted with four vine cuttings per mount. The spacing followed was 60 x 20 cm within and between the rows and the recommended package of practices was adopted. Weeding was done periodically and fertilizers were also applied.

#### Hybridization

#### Cross pollination

The flower buds of female parents were emasculated in the afternoon before anthesis and was covered with butter paper cover. The pollen from anthers of the male parent was dusted on the stigma of female parent or rubbed the anthers on the stigma of female parent. Then the pollinated flowers were covered with a butter paper cover. It was labelled indicating the name of the parents and date of cross-pollination.

#### Harvesting of seeds

From 4 to 6 weeks after the pollination, the matured seeds were collected. The seed capsule was harvested when it became fully brown and the pedicel was dried. The pollinated seeds were collected regularly from the

field and about 300 seeds were obtained from the pollination block.

#### **Viability test**

The separation of well matured seeds from the immature ones was done by putting the seeds into a glass or plastic container containing water. The solution was then stirred in a circular way producing a whirlpool to submerge all seeds. After sometimes, all the floating seeds were discarded by pouring off part of the solution. The seeds that stayed at the bottom were then poured off into a plastic sieve and dried using a paper towel.

#### **Seed scarification**

The hybrid seeds harvested were scarified with concentrated sulphuric acid ( $H_2SO_4$ ) for 10 minutes. The acid was then decanted and the seeds were removed and properly washed with distilled water for several times to



Fig. 1. Sweet potato seeds germinated in the moist petriplate

completely remove the residue of acid. They were kept on a petri plate containing moist filter paper and allowed for germination (Fig.1).

#### **Nursery sowing**

Seeds germinated were planted and maintained in the glass house nursery. After two months, the seedlings were replanted in the field for characterization and tuber flesh colour studies (Fig.2).

#### **Planting of F<sub>1</sub> mapping population**

A total of 200 F<sub>1</sub> progenies were selected for the present study. The vine cuttings were collected from the nursery maintained in ICAR-CTCRI glass house. Each seedling



Fig. 2. Field view of F<sub>1</sub> seedlings

was planted on three mounds with four vine cuttings per mound. The spacing was 60 x 20 cm within and between the rows and the recommended package of practices was adopted.

#### **Phenotyping F<sub>1</sub> seedling population**

The tuber flesh colour variation of F<sub>1</sub> progenies was phenotyped using 1-6 score ranging from white to deep orange viz., score 1 - white, 2 - cream, 3 - yellow, 4 - light orange, 5 - orange and 6 - deep orange. The tuber flesh colours of the progenies were observed visually by taking the cross-section of the interior of the tuber.

#### **Determination of dry matter content**

Sweet potato tubers were freshly harvested and were cut into small pieces. Tubers weighing 50g was taken and dried in hot air oven at 60°C for 72 hrs with three replications. Dry matter content was determined by weighing the initial and final weight, and calculating the percentage of dried weight. The same procedures were followed for all the replications.

$$\text{Dry matter (\%)} = \frac{\text{Dry weight of the tuber}}{\text{Fresh weight of the tuber}} \times 100$$

#### **Determination of starch content**

Starch content was determined based on dry matter content of storage roots. Using a dry weight conversion method, dry matter was measured by the percentage of dry weight to the fresh weight of the storage roots. The conversion formula of the starch content in sweet potato described by Wang, et al. (1989) was followed, i.e.,  $y = 0.86945x - 6.34587$ , in which  $y$  is the starch content and  $x$  is the dry matter content.

### Determination of $\beta$ -carotene

$\beta$ -carotene value was recorded as per the RHS colour chart developed by Burgos, *et al.* (2009) from CIP, Lima, Peru. According to this chart,  $\beta$ -carotene content varied from 0.12 mg/100g FW to 14.37 mg/100g FW.

### Statistical analysis

The data on dry matter and starch content were analyzed by analysis of variance (ANOVA) and the means were compared by Duncan's Multiple Range Test (DMRT) using SAS (9.3). Box plot diagrams were drawn to study the distribution of characters among the progenies.

## Results and Discussion

Sweet potato breeding was difficult because of the polyploidy nature, flowering and seed set, self and cross-incompatibility, heterozygosity and large chromosome number. It is the only natural hexaploid species presently known in the genus in which the hybridization is largely restricted to crosses within the species. Variability within the species is very extensive. Each seedling plant is heterozygous and genetically different from any other (Yen, 1974).

### Tuber flesh colour variation

Sweet potato tuber flesh colour is controlled by the presence or absence, type and amount of pigments present in the internal tissue. Generally observed flesh colours of sweet potato are white, cream, yellow, orange (carotenoids) and purple (anthocyanin) with light, intermediate and dark shades of each. However, some cultivars show purple pigmentation in the flesh in very few scattered spots, pigmented rings or, in some cases, throughout the entire flesh of the root. As the study was conducted using vine cuttings from seedlings, numerous combination of alleles from the parents would have occurred, resulting in the expression of wide variety of morphology (Vimala et al., 2012). In the present study, the progenies of a cross between S-1 (purple skin and white flesh) and ST-14 (cream skin and orange tuber flesh) showed segregation extensively in tuber flesh colour. The progenies exhibiting tuber flesh colour variations includes white, cream, yellow, light orange, orange and deep orange and were categorized on the basis of 1-6 score for phenotypic variation (Table 1). Twenty two per cent of the progenies were orange-fleshed, 22.5% were yellow-fleshed, 15.5% were cream-fleshed, whereas only 8 per

cent constituted white flesh colour which was similar to that of male parent character. Among the OFSP progenies, predominantly observed tuber flesh colour was light orange (28%), orange (22%) and deep orange (4%) colour, respectively. Previous study by Vimala and Binu Hariprakash, (2011) also reported that orange tuber flesh colour, the female parent character was predominant in the progenies (58.8%) and only 8.4% constituted the white flesh colour.

### Dry matter content

The dry matter content of the parent ST-14 was found to be 26.42% and that of S-1 was 37.97%. The dry matter content of the progenies ranged from 18.58% to 45.7%. The box plot data showed an average dry matter content of 32%. Most of the progenies recorded their values for dry matter content between 28% to 35%. The minimum dry matter recorded was 18.58% and the maximum dry matter content recorded was 45.7% (Fig. 3). Among the 200 progenies, 8 per cent showed dry matter content of less than 25% and 24 per centage of the progenies had it above 40%. The progenies having dry matter content above 40% were OW3, OW10, OW15, OW20 and OW91. It was also observed that 22 per cent of  $F_1$  progenies recorded a dry matter almost similar to that of the male parent, whereas 24 percentage of  $F_1$  progenies to that of the female parent.

High dry matter content is one of the major aims in sweet potato breeding programmes. Dry matter content varies due to factors such as variety, location, climate, incidence of pests and diseases, cultural practices and soil types (Jones et al., 1986; Manrique and Hermann, 2000; Shumbusha et al., 2010; Vimala and Hariprakash, 2011). Most genetic studies and the existence of several enzymes involved in starch biosynthesis indicated that dry matter content shows quantitative inheritance (Cervantes-Flores

Table 1. Phenotypic scoring of the mapping population

Sl. No	Score	Tuber flesh colour	No. of progenies	Per cent
1	Score 1	White	16	08.00
2	Score 2	Cream	31	15.50
3	Score 3	Yellow	45	22.50
4	Score 4	Light orange	56	28.00
5	Score 5	Orange	44	22.00
6	Score 6	Deep orange	8	04.00

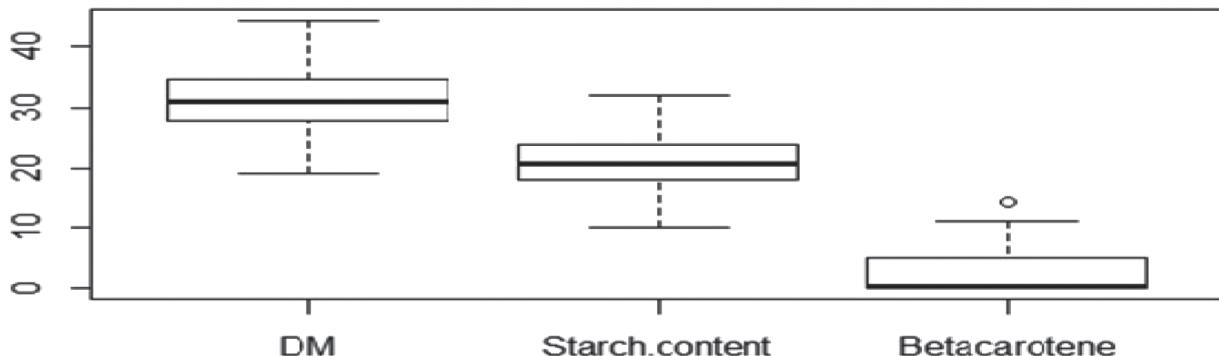


Fig.3. Box plot diagram

et al., 2008). Jones (1986) reported that the value and acceptability of a new sweet potato variety depends on the presence of relevant traits in suitable genetic combination. Generally, orange-fleshed sweet potatoes possess low dry matter content (Simonne et al., 1993), and hence  $\beta$ -carotene and dry matter content are negatively correlated in sweet potato. In the present study, orange-fleshed progenies OW22, OW15, OW98 and OW102 possessed dry matter content of 36, 35, 35.4, 34.6 per cent respectively, but in all the cases, the  $\beta$ -carotene content was found to be low (about 0.12 mg/100g FW). The  $\beta$ -carotene content was also low in white-fleshed as well as in cream and yellow-fleshed sweet potato progenies even though they had high dry matter content. OW5, OW33, OW91, OW157 and OW161 were yellow-fleshed progenies containing low  $\beta$ -carotene content but with high dry matter content of above 40%. The white-fleshed sweet potato progeny OW20 contained 44.38 per cent of dry matter but it had no  $\beta$ -carotene. Therefore, characteristics that meet the preferences of farmers, consumers and market have to be considered in the selection process of new cultivars.

#### Starch content

The total starch content of the parent, ST-14 (16.26%) and S-1 (25.82%) and in the progenies ranged from 9.80 to 32.7%. The data represented in box plot diagram showed the starch content with an average of 22% and the maximum number of progenies had starch content between 19 to 24%. The maximum starch content recorded was 32.7% and minimum 9.80% (Fig. 3). Starch content is directly dependent on the dry matter content of the tuber. The lower the starch content, the lower the dry matter and vice versa. The progeny OW48 recorded the lowest dry matter content of 19.78% with starch

content of 10.85% and the progeny OW157 recorded the highest dry matter content of 45.7% with a starch content of 31.65%. Sixty nine per cent of the progenies showed the starch content below 20%. Twenty two per cent recorded a starch content of above 25% and only 9 per cent were above 30%.

#### $\beta$ -carotene content

The  $\beta$ -carotene content in sweet potato was recorded using RHS colour chart of CIP. According to the RHS colour chart, the female parent, ST-14 recorded a  $\beta$ -carotene content of 14.37 mg/100g FW and the male parent, S-1 had very low carotene in the tuber (0.03 mg/100g FW). Among the 200 progenies, the  $\beta$ -carotene content ranged from 0.03 mg/100g FW – 11.03 mg/100g FW. Among the OFSP progenies, the  $\beta$ -carotene content ranged from 4.92 mg in (OW22) to 7.76 mg/100g FW in the progenies OW21, OW37, OW42, OW47 and OW48 respectively. The highest  $\beta$ -carotene content was recorded in the progenies OW110 and OW113 (11.03 mg/100 FW).

The statistical analysis showed that the  $F_1$  progenies produced by controlled hybridization of ST -14 and S-1 showed high dry matter, starch and  $\beta$ -carotene content. The data reported were the mean of replicates. The accessions for dry matter and starch content were considered statistically significant at 5% level of significance. The mean comparisons were made by the DMRT. This proved the efficiency of controlled hybridization for obtaining new and improved varieties of sweet potato. The progenies, OW137 and OW157 recorded high dry matter as well as high starch content. The dry matter content was above 40% and starch content was above 30%. Among OFSP progenies, OW43 recorded high dry matter content (35%) with high starch (27%).

These hybrid progenies can be further used for breeding programmes and evaluation trial for release of varieties.

## Conclusion

Generally dry matter content above 25% is an important factor for farmers to adopt a new variety of sweet potato. In the present study, the hybrid clone OW37 and OW40 exhibited high dry matter and  $\beta$ -carotene content with a desirable agronomic trait like tuber yield. The carotenoid rich clones also indicated the possibility of significantly improving the nutritive value of sweet potato which will be desirable to the consumers whereas storage roots with high dry matter are more suitable for developing secondary processed foods. Orange-fleshed progenies with high dry matter and starch content can be popularized as an excellent source of  $\beta$ -carotene to prevent vitamin-A deficiency which affects millions of children in the developing countries.

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