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Variation in Crude Protein, Dry Matter and Starch in Inbred and Backcross Lines of Cassava

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Abstract

In cassava, inbred lines are the choice materials for the selection of desirable parents as they reveal a greater amount of additive genetic variance between lines and permit backcrossing for the transfer of desirable traits. Large number of inbred lines of popular cassava varieties and elite clones developed at Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala were screened for desirable traits and used in heterosis breeding programme. Promising backcross hybrids were developed from elite inbred lines as well as two advanced backcross lines of an interspecific hybrid of cassava with *Manihot tristis* having higher crude protein (8-10%) on dry weight basis in their roots. The clones exhibited high variation for crude protein in tubers with a range of 1.11% - 10.40% and 0.37% to 2.74% on dry and fresh weight basis respectively. Of the sixteen high protein clones, four clones *viz.* CPH-75, CPH-105, CPT-88, CPT-109 had crude protein at levels of >2.0% (fresh weight basis). Crude protein content of cassava had high significant negative correlations of -0.4812 and -0.4741 with dry matter and starch contents respectively. Present study revealed high variation for root dry matter (7.57%-49.60%) and starch content (4.01%-42.00%) among inbred lines (S_2) of elite cassava clones. Highly significant positive correlation of 0.8187 was observed between dry matter (DM) percentage and starch in the clonal populations.

Key words : Cassava, crude protein, dry matter, starch, inbreds, *Manihot tristis, correlation*

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important starchy crops grown in the tropical countries. It is well known for its high yield potential and as an efficient carbohydrate producer even under low input conditions. In India, it is grown in the states of Tamil Nadu, Andhra Pradesh, Karnataka & Maharashtra as an important starch yielding industrial crop while it is preferred as a food crop and forms a part of the traditional diet of the people of Kerala and North Eastern states. Cassava is a heterozygous crop adapted to vegetative propagation and its genetic improvement for yield and other agronomic traits have been achieved during the last few decades. Moreover, the inheritance of some of the important traits was also studied earlier (Easwari Amma and Sheela, 1995). Due to the heterozygous nature of the crop, the full spectrum of variation in some of the economic traits like starch content governed by recessive genes could not be fully exploited. However, inbreeding was found to unleash the hidden variability conserved in the heterozygous state in cassava. Cassava roots are rich in starch, but low in protein which is of concern, especially when the diet of poor people lacks protein from other sources with respect to the consumption point of view. There has been much interest in improving the protein content of cassava roots (Bolhius, 1953; Nasser,1999; Chavez et al., 2005; Ceballos et al., 2006), but very little attempts were made in India to improve the protein content and to promote it as a nutritional security crop. In the present study, a wide variation in root dry matter and starch content was generated by inbreeding of elite cassava clones. The variation in crude protein among the promising clones was also studied for the identification of hybrids rich in crude protein content in roots.

Materials and Methods

Inbreeding of elite cassava clones was undertaken at the Central Tuber Crops Research Institute during 2003-2008. Six hundred and seventy eight cassava seedlings and their first clonal progeny were evaluated for root dry matter and starch at nine months after planting. It includes three hundred and sixty four inbred seedlings of S_2 generation of elite clones of cassava, 143 intervarietal hybrids, 105 halfsib progeny, 16 landraces and 50 interspecific backcross hybrids. The inbred lines comprised of second generation inbred families generated through artificial selfing. The backcross hybrids were developed from an interspecific hybrid of cassava with *Manihot tristis* through repeated backcrossing and selection.

Cassava root dry matter content was determined by taking the medial sections of randomly selected peeled tubers and shredded them into thin slices, mixed thoroughly and duplicate samples (50 g) were dried at 65°C for 72 h. The tuber dry matter (gravimetric method), starch (Moorthy et al., (2002) and crude protein (Bailey, 1967) contents were estimated following standard analytical procedures. Crude protein was estimated by multiplying N percentage with the usual constant 6.25 and also by the conversion factor of 3.27 as reported by Hock-Hin and Van-Den (1996). The original conversion factor of 6.25 was maintained to facilitate the comparison with the previous reports. One hundred and twenty six clones were analysed for variation in crude protein content.

Results and Discussion

Crude protein

A total of one hundred and twenty six cassava clones comprising of inbred lines (19), landraces (8), released varieties (7), inter varietal (42) and backcross hybrids (50) were evaluated for crude protein. The clones recorded high variation for crude protein ranging from 1.11% to 10.40% when crude protein (crude protein[a]) was calculated based on multiplying the total nitrogen content (Kjeldahl nitrogen) by a factor of 6.25. For cassava roots, this value may lead to an overestimation of the actual protein content as up to half the nitrogen is believed to be non protein nitrogen. Simmons et al. (2008) reported that cultivated varieties of cassava have high and variable nitrogen contents but low true protein content in roots. Hock-Hin and Van-Den (1996) reported a conversion factor of 3.27 for the better estimate of the cassava root protein content based on Kjeldahl nitrogen. Based on this conversion factor, the crude protein content (crude protein[b]) of the roots of the same cassava clones ranged from 0.57% to 5.39%.

The total population recorded high coefficient of variation of 37.63% for crude protein indicating the possibility for the improvement of this important trait in cassava. Among the different breeding populations evaluated, the landraces and backcross hybrids had the higher coefficient of variation of 47.03% and 43.44% respectively (Table 1.). However, the mean (4.32%) and range (1.99-10.40%) of crude protein content was higher in backcross hybrids as compared to other populations. Higher percentage of root protein content occurs in wild *Manihot* species in comparison to cultivated cassava and Nasser (1999) reported a crude protein content of 7.10%.±0.58 in *Manihot oligandha* Pax. The present study has recorded higher protein content in the progeny derived from *Manihot tristis* as compared to cultivated cassava. Comparison of various characters of the selected accessions was made with the corresponding values of the released varieties

Table 1. Variation for crude protein content in different populations of cassava

Breeding lines	Crude protein [a] on dry weight basis				
	No of lines	Mean (%)	SE	Range (%)	Coefficient of variation (%)
Intervarietal hybrids	42	3.83	0.17	2.21-6.64	27.95
Inbreds	19	3.67	0.23	2.55-6.53	27.27
Released varieties	7	3.14	0.31	1.99-4.21	24.06
Landraces	8	3.49	0.58	1.11-5.87	47.03
Backcross hybrids	50	4.32	0.27	1.99-10.40	43.44
Total population	126	3.95	0.13	1.11-10.40	37.63

Crude protein [a] : Calculated from total nitrogen content based on conversion factor 6.25, SE : Standard error

Table 2. List or best 16 cassava clones having high crude protein content in roots out of total population of 126 clones

Clone	Dry matter (%)	Starch (%)	Crude Protein (%) (D.W. basis[a])	Crude Protein (%) (D.W. basis[b])	Crude Protein (%) (F.W. basis[a])	Crude Protein (%) (F.W. basis[b])	% increase in crude protein over check variety
CPS10	37.88	26.25	5.09	2.64	1.93	1.00	170.29
CPT1	31.44	20.21	5.20	2.70	1.64	0.85	173.99
CPV94	34.08	21.91	5.76	2.98	1.96	1.02	192.50
CPV116	26.86	17.27	5.87	3.04	1.58	0.82	196.20
CPT85	25.72	19.29	5.98	3.10	1.54	0.80	199.91
CPH75	37.56	22.54	6.11	3.17	2.29	1.19	204.35
CPT28	28.36	19.64	6.42	3.33	1.82	0.94	214.71
CPS30	25.58	13.54	6.53	3.39	1.67	0.87	218.42
CPT82	24.42	14.47	6.64	3.44	1.62	0.84	222.12
CPH105	41.25	24.75	6.64	3.44	2.74	1.42	222.12
CPT29	25.72	19.29	6.86	3.56	1.77	0.92	229.52
CPT81	26.76	20.07	6.86	3.56	1.84	0.95	229.52
CPT88	26.70	18.48	7.75	4.02	2.07	1.07	259.14
CPT32	24.06	15.47	8.08	4.19	1.94	1.01	270.24
CPT13	24.06	15.47	8.24	4.27	1.98	1.03	275.43
CPT109	22.28	12.53	10.40	5.39	2.32	1.20	347.98
Mean (selected clones)	28.92	18.82	6.78	3.51	1.92	0.99	
Mean (released varieties)	33.35	24.78	3.14	1.61	1.02	0.55	
t value	NS	3.05**	6.84**	6.84**	5.38**	5.38**	
Sree Jaya (Check variety)	25.88	21.77	2.99	1.55	0.77	0.40	

Crude protein [a] : Calculated from total nitrogen content based on conversion factor 6.25

Crude protein [b] : Calculated from total nitrogen content based on conversion factor 3.27

SE : Standard error, FW : Fresh weight, DW: Dry weight

and then *t* test values are given in Table 2. High significant difference was obtained for crude protein content indicating genetic improvement of this trait.

The crude protein content of 16 clones which were selected for further breeding recorded values above 5.09% (crude protein[a]). Four hybrid clones *viz.*, CPH-75, CPH-105, CPT-88, CPT-109 had high crude protein content (>2.0%) on fresh weight basis. The standard heterosis among those clones was calculated based on the crude protein content of the popular variety Sree Jaya and it ranged from 170.29 - 347.98%. The average dry matter percentage of the selected clones was 28.92%.

In the present study, crude protein content of cassava had a high significant negative correlation of -0.4812 and -0.4741 with dry matter and starch content respectively (Table 3). Hence improvement in crude protein could result in decrease in root DM% and starch %. However, a few clones *viz.*, CPV-94, CPS-10, CPH-75 and CPH-105 had high crude protein content coupled with high dry matter percentage (34.08-41.25%) indicating the possibility of simultaneous selection for root quality traits in cassava through special efforts. The tuber flesh colour did not have any association with protein content. Chavez et al. (2005) recorded high crude protein (5.71-8.75%) in the germplasm accessions of CIAT with a mean of 3.56 and a lower coefficient of variation of 8.72%. The population developed at CTCRI had a mean value of 3.95% with higher coefficient of variation of 37.63%. Nartey (1968) reported that the hydrolytic products of glucosides are incorporated into the amino acids for protein synthesis in cassava. However, Chavez et al. (2005), reported a weak correlation between crude protein content and

Table 3. Correlation of crude protein with dry matter and starch content in cassava.

Traits	Crude protein (%) (D.W. basis)
Dry matter (%)	-0.4812**
Crude protein % (F.W. basis)	0.7399**
Starch % (F.W. basis)	-0.4741**

**Significant at P<0.01; NS non-significant, *Significant at P<0.05

cyanogens in cassava. Cassava roots are a poor source of protein and methionine and lysine are the limiting amino-acids in the root. If cultivars could be bred for the increased quantity of the limiting amino acids, it would enhance the value of the crop as a food and feed. Only about 60 % of total nitrogen in cassava is derived from amino acids and about 1 % of it is in the form of nitrates and hydrocyanic acid. The remaining 38 to 40 % of total nitrogen remains unidentified. The crude protein content of roots appears to be relatively stable and constant with maturity of the plant. The variability for high crude protein content was also reported by Ceballos et al. (2006) that cassava may have up to 8% crude protein content in roots if the standard N to protein conversion factor of 6.25 is used.

The high protein clones reported here will be further evaluated for true protein content, amino acid profile, yield and culinary qualities to evolve a nutritionally rich cassava variety in future.

Dry matter and starch

Increased genetic variability in the different biochemical traits *viz.*, dry matter and starch is essential to promote diversified product development in cassava. In the present study, the cassava lines comprising of inbred lines (D.W. basis), intervarietal hybrids, half sibs and backcross hybrids were evaluated for biochemical traits. The inbred lines showed wide variation with root dry matter ranging from 7.57%-49.60% (Table 4). The highest coefficient of variation in dry matter (26.35%) was recorded by inbred lines followed by back cross lines (20.11%). However, the inbred families tested differed in their variation for dry matter and starch content (Table 5). The highest coefficient of variation (26.22%) for root dry matter was recorded in the inbred family (IC2) developed from a landrace, Ethakkaruppan while the least variation (12.67%) was observed in IL2 developed from Sree Padmanabha. The average dry matter percentage of the population was 35.48%.

The same trend was observed for starch content also with highest variability among inbreds (35.11%) followed by backcross hybrids (27.68%). The percentage of starch content exhibited a wide variation ranging from 4.01 to 42 per cent in the population. The family developed from Ethakkaruppan (IC2) had the highest coefficient of variation (38.91%) for starch content which ranged from 4.01 to 42.0 per cent. The results confirm the increased variability for starch content among inbred lines in cassava. Chavez *et al* (2005), recorded high variation for root dry matter content in cassava with a range of 10.7-57.2 per cent which is in conformity with the present finding. Fifty six clones recorded high starch content (>30%) in the present study.

Correlation studies were conducted to reveal the association of dry matter and starch content of the

Table 4. Variation for dry matter and starch content in different clones of cassava

Breeding lines	No of lines	Dry matter (%)			
		Mean	SE	Range	Coefficient of variation (%)
Intervarietal hybrids	143	38.20	0.68	22.22-49.89	19.06
Inbreds	365	33.50	0.53	7.57-49.60	26.35
Half sib progeny	105	35.79	1.07	14.10-47.62	21.94
Backcross hybrids	50	35.96	1.21	21.34-45.64	20.11
		Starch (% F.W.)			
Intervarietal hybrids	143	23.63	0.54	8.57-39.45	24.41
Inbreds	365	21.17	0.45	4.01-42.00	35.11
Half sib progeny	105	22.54	0.79	9.76-35.17	25.74
Backcross hybrids	50	24.18	1.12	10.06 -35.87	27.68

Table 5. Variation in root dry matter and starch content in different inbred families (S_2) of cassava

Inbred	Root dry matter content (%)				Starch content (%) FW basis			
families	Mean	Range	SE	CV%	Mean	Range	SE	CV%
IA2	38.10	29.96-45.12	0.10	10.46	27.45	20.70-36.90	1.21	17.68
IB2	38.15	12.78-49.34	0.99	18.23	25.96	7.70-37.40	0.90	25.50
IC2	32.48	7.58-48.5	2.01	26.22	21.93	4.01-42.00	2.01	38.91
ID2	39.01	25.58-47.72	1.66	18.03	24.05	13.50-35.70	1.40	24.70
IL2	35.18	30.20-42.90	42.90	12.67	24.48	18.60-35.10	1.60	19.65
Population	35.48	7.58-49.34	0.49	23.08	23.73	4.01-42.00	0.43	30.38

seedlings with their clonal counterpart. Highly significant positive correlation of 0.8187 was observed between dry matter and starch yield in clonal population. The seedling dry matter had significant positive correlation with clonal dry matter content (0.3046**). However, seedling starch content had only positive but non-significant correlation (0.1781) with clonal starch yield. The results indicated the scope for selection of lines having high dry matter thereby improving starch content in cassava. Moreover, inbred lines of elite cassava clones could be the better material for the improvement of starch quality traits in cassava. The elite clones with high root crude protein and starch content identified in the present study can be further used for the population improvement of these two important traits in cassava.

Conclusion

In cassava, variation for starch and crude root protein content has been found to be meager, limiting the genetic improvement of these two important traits. Inbreeding in cassava leads to the expression of recessive alleles conserved in heterozygous state. The present study highlights the release of a wide spectrum of variability for starch content in cassava by the development of inbred families of elite clones. Fifty six high starch (>30%) clones were identified for further starch improvement programmes. Since all the released varieties were having low root crude protein content, attempt was made to develop hybrids from inbred families and advanced backcross lines with high protein gene from *M.tristis*. High variability for crude protein led to the identification of hybrids with high root crude protein (>2% on FW basis) which will be further tested for true protein content to strengthen the cassava biofortification programmes in India.

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