Identification of regions of cassava genome associated with increased carotenoids content in roots

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Introduction

Effects of vitamin A deficiency ranges from night blindness to those of xerophthalmia and keratomalacia, leading to total blindness. The principal vitamin A source is from animal origin, this has an elevated cost for poor people. The pro-vitamin A carotenoids are cheaper source since they are found abundantly in plants. Cassava plays an important role as main source of low-cost energy for 800 million of the world's poorest people, but is not an important source of other nutrients such iron, zinc, and vitamin A[1]. It has been demonstrated that cassava germplasm with yellow roots may contribute significantly to resolve problem of Vitamin A deficiency in poor countries. We proposed to find molecular markers tightly linked with high content of ß-carotene to assist accelerating the breeding for improved levels of ß-carotene in cultivated cassava.

Materials and methods

Plant material: thirty-two segregating families (756 ind.) derived from reciprocal crosses between yellow, cream and white roots were grown and evaluated for total carotene content.
Extraction and quantification of carotenoids: the methodology follows standard protocols developed by HarvesPlus[2].
Bulk segregant analysis (BSA): To find tightly linked markers with high content of ß-carotene, 800 microsatellites (SSRs) were screen between two contrasting bulks (12 individuals each) for carotene content.
Genetic Mapping: A genetic linkage map was constructed for cassava using MapMaker/EXP version 3.0b[3]. Locus orders were inferred with a LOD score of 3.0 using a log-likelihood threshold of 2.0 and then using a log-likelihood threshold of 1.0.

Results

Table 1 show 5 of the 32 families selected for the bulk segregant analysis (BSA). This lines were selected based on two criteria [high carotenoid content and size of progeny (>80)]. The bulks were selected as shown in Figure 1. The results of the BSA analysis showed that SSR markers **NS-717**, **SSRY-313 and SSRY-251** had the highest degree of association with high carotene content (Table 2). For the QTL analysis, 229 individuals of the segregating population COL1684 x MTAI1 were genotyped using 140 SSR markers and the resulting genetic map had 25 linkage groups with a LOD of 4.0. The average distance between markers is 21.5 cM covering 1,500 cM (Figure 2). The linear regression analysis of the phenotypic and genotypic data using MAPMAKER\QTL (Figure 3), showed that **NS-717**, **SSRY-313 and SSRY-251** were associated with three major QTLs for high carotene content (Table 3).

Table1. Correlation analysis between contrasting bulks (Yellow vs. White roots)

GM708		GM734		CM9816	
Marker	% Correlation	Marker	% Correlation	Marker	% Correlation
SSRY-324	14	SSRY-200	10	***SSRY-324***	23
SSRY-66	16	SSRY-9	10	**SSRY-66**	28
SSRY-226	23	NS-267	10	SSRY-242	30
NS-267	26	SSRY-178	11	SSRY-172	33
SSRY-9	27	NS-119	14	SSRY-251	35
SSRY-242	28	NS-53	17	SSRY-330	37
SSRY-178	31	SSRY-21	18	***NS-717***	41
SSRY-88	31	SSRY-66	27	SSRY-9	42
NS-717	32	SSRY-242	31	SSRY-195	42
SSRY-251	42	***SSRY-313***	35	NS-158	43
SSRY-313	44	***NS-717***	41	***SSRY-313***	47
		SSRY-251	51		

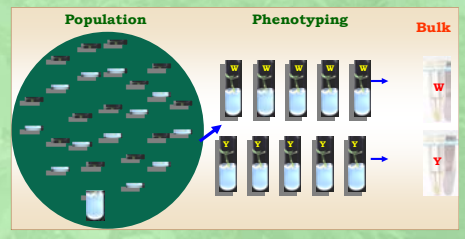

Figure 1. Bulk segregant analysis

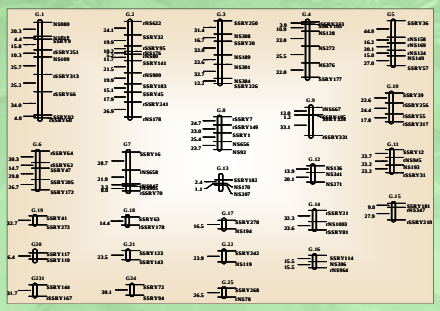

Figure 2. Cassava genetic linkage map.

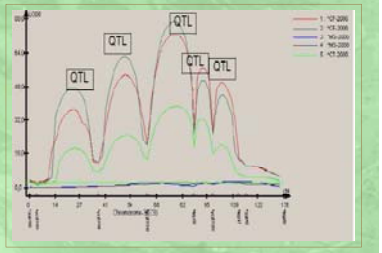

Figure 3. QTL mapping for high carotene content

Table 1. Segregating families with high content of carotenoids.

Crosses	Female	Root colour	Male	Root colour	N° individuals	Total carotenoids (ug/g FW)
CM 9816	MCOL 2295	Yellow	SM 980-4	Cream	95	4.83
GM 705	MBRA 1A	Yellow	MCOL 1734	Cream	105	5.13
GM 708	MBRA 1A	Yellow	MMAL 66	Cream	80	3.14
GM 734	MTAI2	Cream	CM 3750-5	White	85	3.62
GM 893	SM 805-15	White	MPER 297	Cream	108	3.10

Table 3. Composite interval analysis for three carotene characteristics.

Caracteristica	QTL	Intervalo SSR	G.L	LOD
CCT	QCCT1	rSSRY66-rSSRY313	1	8
	QCCT2	rSSRY313-NS109	1	33
	QCCT3	NS109-NS717	1	17
CPR-2006	QCPR6-1	rSSRY66-rSSRY313	1	28
	QCPR6-2	rSSRY313-NS109	1	68
	QCPR6-3	NS109-rSSRY251	1	34
	QCPR6-4	rSSRY251-NS717	1	30
CPR-2008	QCPR8-1	rSSRY66-rSSRY313	1	33
	QCPR8-2	rSSRY313-NS109	1	78
	QCPR8-3	NS109-rSSRY251	1	31
	QCPR8-4	rSSRY251-NS717	1	28

Conclusions

The BSA and the QTL analysis allow the identification of three SSR (**NS-717**, **SSRY-313 and SSRY-251)** putative associated with high carotene content. These three markers are all located on linkage group D of the cassava genetic map.

References

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[2] Rodríguez-Amaya DB, Kimura M. 2004. HarvestPlus Handbook for carotenoid analysis. HarvestPlus Technical Monograph 2. Washington, DC and Cali. International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).
[3] Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage map of experimental and natural families. Genomics 1:174-181.