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The inheritance of β-amylase null in storage roots of sweet potato, *Ipomoea batatas* (L.) Lam.

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Summary. Several sweet potato genotypes were found to lack completely or to have only traces of β-amylase in their storage roots. Such genotypes do not increase in sweetness during cooking because, without a sufficient amount of β-amylase, the normal hydrolysis of starch to maltose does not occur in the cooking process. In order to study the inheritance of this biochemical variant in the genotype, 41 families were generated. The following conclusions were drawn from analyzing these families. (1) This trait is controlled by one recessive allele (designated *β-amy*) (2) It is inherited in a hexasomic or tetradisomic manner, but not disomically or tetrasomically. This conclusion supports previous cytological data that sweet potato is an autohexaploid or has two identical genomes plus one genome which is somewhat different. (3) The *β-amy* allele appears to exist at a high frequency in cultivated germplasm. (4) Breeding sweet potato for low β-amylase activity is relatively easy. New types of sweet potato without normal β-amylase activity have great potential for processing and as a staple food.

Key words: *Ipomoea batatas* – Sweet potato – β-Amylase – Polysomic inheritance – Hexaploid

Introduction

The sweet potato, *Ipomoea batatas* (L.) Lam., is valuable because it is a rural, low-input crop that can produce stable yields under suboptimal conditions. In developing countries, the sweet potato ranks fifth among major food crops in terms of total production and economic value, and sixth in dry matter production. As a contributor of energy and protein to the human diet it ranks seventh and ninth, respectively (Gregory et al. 1988). The sweet potato is especially important in the cropping systems and diets of poor people in tropical areas.

Among the components of sweet potato storage roots are starch and the enzyme, β-amylase. When the root is cooked, the starch is hydrolyzed to maltose by β-amylase and, consequently, the sweetness increases dramatically (Gore 1923). Until fairly recently, it was assumed that all sweet potato storage roots had β-amylase activity and that their sweetness increased during cooking. However, in 1983, Martin and Ruberte reported a new type of sweet potato that had low β-amylase activity and the taste of which was only slightly sweet when boiled.

In 1987, the new sweet potato variety Satsumahikari was registered in Japan (Kukimura et al. 1988). Similarly, this variety has null or low β-amylase activity and its maltose content does not increase in cooking (Baba et al. 1987b). Using this new variety, several sweet potato processed products such as sweet potato flakes and granules, fried chips, and frozen french fries are being developed (Kukimura et al. 1988). The new type of non-sweet sweet potato can be eaten every day as a staple food (Martin and Ruberte 1983; Martin 1987). Thus, lack or very low activity of β-amylase will make it possible for this traditional crop to be used in non traditional ways (i.e., processed) and also as a staple food.

The experiments reported in this paper were conducted to elucidate the inheritance of this null mutant (i.e., with null or low activity of β-amylase), which is an important trait for sweet potato improvement. Furthermore, two rapid methods for classifying sweet potato genotypes into two distinct groups (normal versus null β-amylase) were investigated, to determine whether such simple methods can be effectively utilized.

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Materials and methods

Development and use of rapid methods for detecting null mutants

Five Japanese cultivars and 242 hybrid seedlings derived from these varieties were used in our experiments. Koganesengan, Kokei-14, and Beniazuma are leading Japanese cultivars with normal β-amylase activity in storage roots, while Satsumahikari and Kanto-92 have only traces or null amounts of β-amylase (Baba et al. 1987b). Four hybrid seedling families were produced by crossing the β-amylase null Satsumahikari with the four cultivars mentioned above.

Crosses were made by hand-pollinations in the greenhouse at the Ibusuki Branch, Kyushu National Agricultural Experiment Station, during the spring of 1984. The storage roots of the cultivars and seedlings were harvested in the fall of 1985 at Ibusuki and used for β-amylase analysis.

Freshly harvested storage roots were peeled, grated, and squeezed to get crude extracts, which were then centrifuged at 12,000 rpm for 10 min at 4 °C. Supernatants were dialyzed using cellophane tubes at 3 °C overnight to get extracts. β-Amylase activity was determined by using these extracts according to the method of Takeda and Hizukuri (1972). One unit of β-amylase activity was defined as a generation of 1 μM maltose per minute per 1 ml of extract.

A rapid test for determining β-amylase activity in storage roots was developed and used for screening a large number of seedlings. The procedure is as follows. Crude extracts were centrifuged at 3,000 rpm for 10 min. The 0.05 ml of the supernatant was pipetted into two test tubes: test tube "A" with 0.5 ml of 2% soluble starch containing 0.05 M acetate buffer, pH 4.8, and test tube "B" with 0.5 ml of water. The test tubes were incubated for 10 min at 37 °C in a water bath shaker to allow the β-amylase in the extracts to hydrolyze the soluble starch to maltose. A tablet of reagent for the testing of reducing sugars, RESUTER (Shibata Scientific Technology Ltd., Tokyo), was added to each test tube. The reducing sugar index (RSI) was judged by the simple observation of comparing the color of the reacting solution with the color reference standard. If the RSIs of two test tubes were the same, the genotype examined did not have a sufficiently high level of β-amylase activity to be able to hydrolyze starch to maltose. If the RSIs of two test tubes were different and the RSI of tube "A" with starch was higher than the RSI of tube "B", the genotype examined exhibited β-amylase activity.

Since RESUTER might not be available in some countries and since it costs about US$ 0.50 to test each sample, another method using Fehling's solution was developed. The procedure was identical to that used for RESUTER up to the step of incubation at 37 °C. Instead of RESUTER, 0.05 ml of Fehling's solution was added to each incubated test tube, and then the solutions were gently boiled. In the presence of reducing sugars, copper oxide (Cu_2O) was generated and precipitated. Therefore, the color of the reacting solution changed from blue (bluish red) to orange or red. If the reacting solution of the two tubes had the same color, the genotype examined was regarded as not having normal β-amylase activity. If the reacting solution of tube "A" with starch was orange (or red) in color, and test tube "B" with water was blue, the genotype examined was considered to have normal β-amylase activity.

Inheritance study experiments

Nine cultivars or advanced breeding stocks derived from Japanese breeding programs had been previously identified as having null or very low β-amylase activity. Fourteen cultivars or advanced breeding stocks with normal β-amylase activity were used for crosses. Three of these normal cultivars (Tenian, Capela, and L-4-5) were introduced from other countries, and the rest represent leading Japanese cultivars or advanced breeding stocks. Using the 9 null mutants and the 14 normal clones, 41 families were generated. The first 17 families represented crosses between null mutant parents (Kanto-92, Satsumahikari, 84 180-20, 84 180-75, Ibaraki-1, 840P3-1, 840P9-3, Kyukei-116) or were the result of selfing the null mutant clone, Kyukei-84 028-2. The next 21 families were produced by crossing null mutants with normal clones. The last 3 families were obtained by crossing normal clones with other normal clones or by selfing normal clones (see Table 5 for detail).

The crosses were made by hand-pollinations in the greenhouse at the Ibusuki Branch, Kyushu National Agricultural Experiment Station, during the spring of 1984 and the fall of 1985 and 1986. The 1,876 seedlings derived from 41 families were grown in the field of the Ibusuki Branch during the summers of 1985, 1986, and 1987, and in the greenhouse during the winter of 1987. Storage roots harvested from the seedlings were analyzed for β-amylase activity by the simple methods previously described. These methods allowed us to classify the large number of progenies into two classes: normal or null mutant.

Expected theoretical ratios

Cytological information indicates that the sweet potato has three genomes, and that two of these three are probably identical to each other and the third is slightly different from them (Jones 1965; Ting and Kehr 1953; Shiotani and Kawase 1987). Shiotani and Kawase (1987) postulated the genome constitution of the sweet potato as $B_1 B_1 B_2 B_2 B_2 B_2$, but the degree of homology between B_1 and B_2 genomes could not be estimated with accuracy. Therefore, as clearly pointed out by Jones (1967), four models of inheritance are possible in the sweet potato: hexasomic, tetradisomic, tetrasomic, and disomic. Thus, the following four hypotheses were developed. Since frequency of multivalent formation is low in the sweet potato (Jones 1965), we assumed no double reduction for the development of our hypothesis. Another important assumption was that null or low activity of β-amylase was controlled by one recessive gene.

Hypotheses

Hypothesis I ($B_1 = B_2$, hexasomic inheritance). Under this hypothesis, there is no preferential pairing among the six homologous chromosomes and, thus, hexasomic inheritance occurs. We assume that the lack of β-amylase activity is due to a single recessive mutant allele, β-*amy*. The genotypes of β-amylase null are β-*amy* β-*amy* β-*amy* β-*amy* β-*amy* β-*amy* ($\beta\text{-}amy^6$, for short) and genotypes of normal varieites are $\beta\text{-}Amy^1$ $\beta\text{-}amy^5$, $\beta\text{-}Amy^2$ $\beta\text{-}amy^4$, $\beta\text{-}Amy^3$ $\beta\text{-}amy^3$, $\beta\text{-}Amy^4$ $\beta\text{-}amy^2$, $\beta\text{-}Amy^5$ $\beta\text{-}amy^1$, or $\beta\text{-}Amy^6$. The gametic ratios expected from each of these genotypes are presented in Table 1. Thus, all gametes of β-amylase null varieties would be $\beta\text{-}amy^3$, and the ratio of normal to mutant gametes of normal varieties having the $\beta\text{-}Amy^1$ $\beta\text{-}amy^5$ genotype would be 1 $\beta\text{-}Amy^1$ $\beta\text{-}amy^2$: 1 $\beta\text{-}amy^3$, and so on. The segregation ratio from crosses between this genotype and a mutant variety is 1 $\beta\text{-}Amy^1$ $\beta\text{-}amy^5$: 1 $\beta\text{-}amy^6$, or 1 normal:1 mutant. The possible segregation ratios of normal versus β-amylase null phenotypes for testcrosses involving the various normal genotypes under this hypothesis are 1 : 1, 4 : 1, 19 : 1, and 1 : 0 (last column in Table 1).

Hypothesis II (tetradisomic inheritance). Under this hypothesis, we assume that the B_1 and B_2 genomes have strict preferential pairing and, thus, genes located in the B_1 genome would have disomic inheritance and those in the B_2 genome would have tetrasomic inheritance. Alleles controlling β-amylase are designated as $\beta\text{-}Amy_1$ and $\beta\text{-}amy_1$ in the B_1 genome, and $\beta\text{-}Amy_2$ and $\beta\text{-}amy_2$ in B_2. There is no phenotypic difference between the two

Table 1. Genetic hypotheses for the root β-amylase in sweet potato. Also note that A, a, A_1, A_2 and a_2, indicate β-*Amy*, β-*amy*, β-Amy_1, β-Amy_2, and β-amy_2, respectively. The last column shows the expected segregation ratio (normal: low β-amylase) when a normal type is crossed with a null type (testcross)

Hypothesis	Phenotype	Genotype	Gametic output	Expected ratio from testcross (normal:low β-amylase)
I	–	β-amy^6	a^3	
	+	β-Amy^1 β-amy^5	$1A^1a^2 : 1a^3$	1:1
		β-Amy^2 β-amy^4	$1A^2a^1 : 3A^1a^2 : 1a^3$	4:1
		β-Amy^3 β-amy^3	$1A^3 : 9A^2a^1 : 9A^1a^2 : 1a^3$	19:1
		β-Amy^4 β-amy^2	$1A^3 : 3A^2a^1 : 1A^1a^2$	1:0
		β-Amy^5 β-amy^1	$1A^3 : 1A^2a^1$	1:0
		β-Amy^6	A^3	1:0
II	–	β-amy_1^2 β-amy_2^4	$a_1^1a_2^2$	
	+	β-Amy_1^1 β-amy_1^1 β-amy_2^4	$1A_1^1a_2^2 : 1a_1^1a_2^2$	1:1
		β-Amy_1^2 β-amy_2^4	$A_1^1a_2^2$	1:0
		β-amy_1^2 β-Amy_2^1 β-amy_2^3	$1a_1^1A_2^1a_2^1 : 1a_1^1a_2^2$	1:1
		β-Amy_1^1 β-amy_1^1 β-Amy_2^1 β-amy_2^3	$1A_1^1A_2^1a_2^1 : 1a_1^1A_2^1a_2^1 : 1A_1^1a_2^2 : 1a_2^2a_2^2$	3:1
		β-Amy_1^2 β-Amy_2^1 β-amy_2^3	$1A_1^1A_2^1a_2^1 : 1A_1^1a_2^2$	1:0
		β-amy_1^2 β-Amy_2^2 β-amy_2^2	$1a_1^1A_2^2 : 4a_1^1A_2^2a_2^1 : 1a_1^1a_2^2$	5:1
		β-Amy_1^1 β-amy_1^1 β-Amy_2^2 β-amy_2^2	$1A_1^1A_2^2 : 1a_1^1A_2^1 : 4A_1^1A_2^1a_2^1 : 4a_1^1A_2^1a_2^1 : 1A_1^1a_2^2 : 1a_1^1a_2^2$	11:1
		β-Amy_2^1 β-Amy_2^2 β-amy_2^2	$1A_1^1A_2^2 : 4A_1^1A_2^1a_2^1 : 1A_1^1a_2^2$	5:1
		β-amy_1^2 β-Amy_2^3 β-amy_2^1	$1a_1^1A_2^2 : 1a_1^1A_2^1a_2^1$	1:0
		β-Amy_1^1 β-amy_1^1 β-Amy_2^3 β-amy_2^1	$1A_1^1A_2^2 : 1a_1^1A_2^2 : 1A_1^1A_2^2a_2^1 : 1a_1^1A_2^1a_2^1$	1:0
		β-Amy_1^2 β-Amy_2^3 β-amy_2^1	$1A_1^1A_2^2 : 1A_1^1A_2^1a_2^1$	1:0
		β-amy_1^2 β-Amy_2^4	$a_1^1A_2^2$	1:0
		β-Amy_1^1 β-amy_1^1 β-Amy_2^4	$1A_1^1A_2^2 : 1a_1^1A_2^2$	1:0
		β-Amy_1^2 β-Amy_2^4	$A_1^1A_2^2$	1:0
III	–	β-amy_2^4	a_2^2	
	+	β-Amy_2^1 β-amy_2^3	$1A_2^1a_2^1 : 1a_2^2$	1:1
		β-Amy_2^2 β-amy_2^2	$1A_2^2 : 4A_2^1a_2^1 : 1a_2^2$	5:1
		β-Amy_2^3 β-amy_2^1	$1A_2^2 : 1A_2^1a_2^1$	1:0
		β-Amy_2^4	A_2^2	1:0
IV	–	β-amy_1^2	a_1^1	
	+	β-Amy_1^1 β-amy_1^1	$1A_1^1 : 1a_1^1$	1:1
		β-Amy_1^2	A_1^1	1:0

+, – indicate normal and null β-amylase, respectively

dominant genes, β-Amy_1 and β-Amy_2. The genotype of mutant varieties would be β-amy_1 β-amy_1, β-amy_2 β-amy_2 β-amy_2 β-amy_2 (β-$amy_1{}^2$, β-$amy_2{}^4$). All gametes of these varieties would be β-$amy_1{}^1$, β-$amy_2{}^2$, and genotypes of all hybrid seedlings between these varieties would be β-$amy_1{}^2$ β-$amy_2{}^4$, so all seedlings would have null or low activity. The possible genotypes of normal varieties and the expected gamete segregations of these varieties are shown in Table 1 (Hypothesis II). Thus, the possible phenotypic ratios of normal: mutant seedlings from testcrosses between normal and mutant varieties are 1:1, 3:1, 5:1, 11:1, and 1:0 (last column in Table 1).

Hypothesis III (tetrasomic inheritance). Here we assume that the alleles for β-amylase are β-Amy_2 and β-amy_2, located in the genome B_2. The genotypes of normal and mutant varieties and gamete segregation expected for these varieties are shown in Table 1 (Hypothesis III). The expected phenotypic ratios of hybrid progenies between normal and mutant varieties are 1:1, 5:1, and 1:0 (last column in Table 1).

Hypothesis IV (disomic inheritance). We assume that the alleles for β-amylase are β-Amy_1 and β-amy_1, located in genome B_1. The genotypes of normal and mutant class varieties and gamete segregations expected for them are shown in Table 1 (Hypothesis IV). The possible phenotypic ratios of hybrid progenies between normal and mutant varieties are 1:1 and 1:0 (last column in Table 1).

Results

Results of root β-amylase activity in the 242 seedlings of the four families analyzed by the method of Takeda and Hizukuri (1972) are summarized in Table 2. The values ranged from zero (null or not detected) to 1,668 units. It became clear that the seedlings could be classified into two distinct groups. The first group had null or a very

Table 2. Segregation of root β-amylase activaties in four seedling families as analyzed by the method of Takeda and Hizukuri (1972)

Cross		No. of seedlings evaluated	No. of seedlings with different levels of β-amylase activities			
Female	Male		0~9.9[a]	10.0~99.9[b]	100.0~999.9	>1,000
Satsumahikari	× Beniazuma	86	5	8	65	8
Satsumahikari	× Koganesengan	40	10	5	25	0
Kokei-14	× Satsumahikari	57	2	5	48	2
Kanto-92	× Satsumahikari	59	59	0	0	0

[a] Maximum value of this group was 1.50 units
[b] Minimum value of this group was 36.70 units

Table 3. β-amylase activity of three sweet potato cultivars measured by three different methods

Cultivar	β-Amylase activities[a] (unit)	Resuter (RSI[b])		Fehling's solution	
		A[c]	B[c]	A[c]	B[c]
Beniazuma	1,220	0.6	0.2	Red	Bluish red
Satsumahikari	0.11	0.08	0.2	Redish blue	Redish blue
Kanto-92	0.22	0.08	0.05	Redish blue	Redish blue

[a] Measured by the method of Takeda and Hizukuri (1972)
[b] RSI – reducing sugar index
[c] A – Test tube with 2% soluble starch. B – test tube with water

low level of β-amylase activity with a maximum of only 1.5 units, which was found in one of the seedlings of the Satsumahikari × Koganesengan cross. The numbers of seedlings of this group in the four families were 5, 10, 2, and 59, respectively. The second group had a high level of β-amylase activity ranging from 36.7 units (the lowest in this group) to 1,668 units. It was also noted that the four families differed in the ratios of segregants. Both Kanto-92 and Satsumahikari had only a trace amount of β-amylase (Table 3), and all of their hybrids' progenies had null or a low level (maximum was 1.27 unit) of β-amylase activity (Table 2). The other families, which are crosses between Satsumahikari (β-amylase null) and three leading cultivars (normal β-amylase activity), had two groups of progenies; clear segregations were observed.

Baba et al. (1987a) studied the effects of microwave irradiation on dehydrated sweet potato flour. When β-amylase activity was reduced to the level of about 5 units by the irradiation, the flour did not show an increase in its maltose content after regular cooking. This indicated that 5 units may represent the threshold for normal conversion of starch into maltose. Thus, it appears that our classifying of seedlings with β-amylase activity up to 1.50 units into the "null" group was validated.

The values obtained by the simple methods (RESUTER and Fehling's solution) were checked by measuring β-amylase activity in three cultivars (Table 3). Beniazuma as measured by Takeda and Hizukuri (1972) had a high (1,220 units) level of β-amylase, and the rapid test methods also indicated normal activities of β-amylase in this genotype (a threefold increase of RSI in the RESUTER tests and a change of color in the Fehling's solution tests). Both Satsumahikari and Kanto-92 had only trace amounts of β-amylase as measured by the method of Takeda and Hizukuri (1972), and the rapid test method indicated null or very low activity of β-amylase.

The validity of the two methods of classifying sweet potato genotypes into two classes (normal β-amylase versus null β-amylase) was further tested by using three segregating families. The seedlings were first analyzed by the method of Takeda and Hizukuri (1972) and classified into two groups: β-amylase null and normal β-amylase. Thereafter, the two simple methods were applied and the seedlings were classified into the normal or null categories. Complete agreement of the results obtained by the three methods was obtained in the 114 seedlings of the three families (Table 4).

The 17 families derived from crosses between null parents produced only null class progenies (Table 5). None of the 545 progenies was classified as normal. The next 21 families (family nos. 18–38) represent crosses between normal and null parents. Except for the last 2 families, all had segregation ratios for normal and null classes with varying frequencies of null class segregants. Families 37 and 38 had no "null" segregants.

Family 39 was a cross between normal class parents, and still segregated null class progenies. The genotypes of both parents of cross 39 were estimated from the results

Table 4. Comparison of β-amylase activity (normal or null) measured by three methods using three segregating families

Cross	No. of seedlings checked	No. of seedlings normal (+) or null (−) β-amylase	Results of	
			Resuter	Fehling's solution
Satsumahikari × Koganesengan	35	25 (+) 10 (−)	All + All −	All + All −
Kokei-14 × Satsumahikari	48	46 (+) 2 (−)	All + All −	All + All −
Kanto-92 × Satsumahikari	31	31 (−)	All −	All −

of families 21, 22, and 29, and the expected ratios for family 39 were calculated and tested. The last two families (40 and 41) were produced by selfing. The parental clones of families 40 and 41 were assumed to have only one dominant allele based on the results of families 19 and 20, and the expected ratios were calculated and tested.

The results of all 21 testcrosses (families 18 through 38) were well explained by either Hypothesis I or II. However, while Hypothesis III and Hypothesis IV could explain some of the families, there were 4 families (nos. 27, 31, 32, and 35) that Hypothesis III could not explain, and Hypothesis IV failed to explain the segregation ratios of 17 families.

No reciprocal differences in segregation of this trait were observed. There were three pairs of reciprocal crosses (families 21 and 22; 24 and 25; 34 and 35) in our experiments, and each of them had similar segregation ratios.

Discussion

The method of Takeda and Hizukuri (1972) gives a precise and quantitative measurement of β-amylase activity but, since a breeder is interested in screening a large number of genotypes for this qualitative trait (normal versus β-amylase null), simpler methods may be found to have practical value, even though they are not so precise. The two methods we evaluated were found to be quick and still sufficiently reliable for classifying genotypes into two categories (normal versus β-amylase null).

The following assumptions were set forth to propose an inheritance mode for low β-amylase activity.

Assumptions

Nonoccurrence of double reduction. Although double reduction may occur in the sweet potato, we assumed that it only marginally influenced our segregating ratios. Cytological data indicate, in fact, that the frequency of multivalent formation is rather low in this plant.

Qualitative versus quantitative inheritance of β-amylase activity. We assumed that the trait of null β-amylase is a qualitative character controlled by a single gene. The results of β-amylase activity in the 242 seedlings revealed two discrete groups: one with β-amylase of 36.7 to 1,668 units, and the other with null to 1.5 units of β-amylase activity (Table 3). Although there are other factors which may influence the specific degree of β-amylase activity in a given genotype, the low level of β-amylase activity found in Satsumahikari and other genotypes, which do not become sweet even after cooking, is controlled in a qualitative manner.

Dominant versus recessive inheritance of null β-amylase level. The segregation of only null types in the 545 progenies of the first 17 families (Table 5), which are progenies of null-type parents, strongly supports the hypothesis that this trait is recessive. If this "null" trait had been dominant, all null-type genotypes would have at least one dominant allele. This, in turn, would mean that at least 50% of progenies derived from null would be null, regardless of the possible type of inheritance (disomic, tetrasomic, tetrasomic, hexasomic). There were many families in which the frequency of null types was significantly less than 50% (families 37 and 38 are good examples).

A single recessive gene versus several recessive genes. We assumed that this null trait is controlled by a single recessive gene and then tried to explain the segregation ratios obtained. Assuming that two recessive genes are required for the null type, genotypes with aabb would be null, and any genotype with at least one dominant allele of A or B would be normal. The expected segregation ratios from crosses between normal and null would be 1:0, 1:1, and 3:1 under Hypothesis IV. The segregation ratios of families 30–36 significantly deviated from 3:1. However, if we further assume an involvement of three recessive genes, all segregation ratios of Table 5 can be explained even under Hypothesis IV. Nonetheless, the simplest model should be used as long as it works to explain the results

Table 5. Segregation of root β-amylase activity *in* 41 seedlings families and test of segregation ratios under four hypotheses. "+" and "−" indicate normal and null β-amylase, respectively

Family no.	Pedigree			No. of plants		Hypothesis I		Hypothesis II		Hypothesis III		Hypothesis IV	
				β-Amylase activity		Ratio tested	χ^2	Ratio tested	χ^2	Ratio tested	χ^2	Ratio tested	χ^2
				+	−								
1–17	See text		− × −	0	545								
18	Capela	× Satsumahikari	+ × −	25	29	1:1	0.30	1:1	0.30	1:1	0.30	1:1	0.30
19	Satsumahikari	× 84180-32	− × +	36	33	1:1	0.13	1:1	0.13	1:1	0.13	1:1	0.13
20	Satsumahikari	× 84180-48	− × +	25	18	1:1	1.14	1:1	1.14	1:1	1.14	1:1	1.14
21	Koganesengan	× Satsumahikari	+ × −	33	5	4:1	1.11	3:1	2.84	5:1	0.34	1:1	20.63**
								5:1	0.34				
								11:1	1.16				
22	Satsumahikari	× Koganesengan	− × +	30	10	4:1	0.63	3:1	0.00	5:1	2.00	1:1	10.08**
								5:1	2.00				
23	Beniwase	× Satsumahikari	+ × −	34	6	4:1	0.63	3:1	2.13	5:1	0.08	1:1	19.60**
								5:1	0.08				
								11:1	2.33				
24	Kanto-98	× Satsumahikari	+ × −	47	11	4:1	0.04	3:1	1.13	5:1	0.22	1:1	22.34**
								5:1	0.22				
25	Satsumahikari	× Kanto-98	− × +	42	11	4:1	0.02	3:1	0.51	5:1	0.64	1:1	18.13**
								5:1	0.64				
26	84180-20	× Kanto-98	− × +	45	14	4:1	0.51	3:1	0.05	5:1	2.12	1:1	16.29**
								5:1	2.12				
27	Kyukei 78187-2	× Satsumahikari	+ × −	27	11	4:1	1,90	3:1	0.32	5:1	4.13*	1:1	6.74**
28	Kyukei 78187-2	× Kyukei 84028-2	+ × −	47	11	4:1	0.04	3:1	1.13	5:1	0.22	1:1	22.34**
								5:1	0.22				
29	Tinian	× Satsumahikari	+ × −	78	16	4:1	0.52	3:1	3.19	5:1	0.01	1:1	40.89**
								5:1	0.01				
30	Beniazuma	× Satsumahikari	+ × −	35	3	4:1	3.48	5:1	2.11	5:1	2.11	1:1	26.95**
						19:1	0.67	11:1	0.01				
31	Satsumahikari	× Beniazuma	− × +	99	6	19:1	0.11	11:1	0.94	5:1	9.07**	1:1	82.37**
32	Kokei-14	× Satsumahikari	+ × −	58	2	19:1	0.35	11:1	1.96	5:1	7.68**	1:1	52.27**
33	Kokei-14	× Kyukei-116	+ × −	47	5	4:1	3.50	5:1	1.86	5:1	1.86	1:1	33.92**
						19:1	2.33	11:1	0.11				
34	Kyushu-59	× Kyukei-116	+ × −	53	4	19:1	0.49	5:1	3.82	5:1	3.82	1:1	42.12**
								11:1	0.13				
35	Kyukei-116	× Kyushu-58	− × +	52	3	19:1	0.02	11:1	0.60	5:1	4.98*	1:1	43.65**
36	Nohrin-5	× Satsumahikari	+ × −	48	4	19:1	0.79	5:1	3.02	5:1	3.02	1:1	37.23**
								11:1	0.03				
37	Chikei 682-11	× Satsumahikari	+ × −	78	0								
38	L-4-5	× Satsumahikari	+ × −	78	0								
39	Koganesengan	× Tinian	+ × +	51	5	24:1	3.54	15:1	0.69	24:1	3.54	3:1	7.71**
								23:1	3.18				
40	84180-32	Selfing	+ × +	47	11	3:1	1.13	3:1	1.13	3:1	1.13	3:1	1.13
41	84180-48	Selfing	+ × +	21	4	3:1	1.08	3:1	1.08	3:1	1.08	3:1	1.08

*,** Significant deviation from ratio tested at $p=0.05$ and $p=0.01$ levels, respectively

obtained. In other crops such as barley (Kreis et al. 1987) and soybean (Hildebrand and Hymowitz 1980), lack of β-amylase has been shown to be controlled by a single recessive mutant.

Best hypotheses

The results of the 41 families are best explained by both Hypotheses I and II. Hypothesis IV failed, in fact, to explain more than half of the families we tested, therefore, it was rejected. Similarly, Hypothesis III is not appropriate for explaining the results of four families. It appears that either Hypothesis I or II should be accepted as the most appropriate model, as long as the preceding assumptions hold true.

Previous cytological studies indicated that sweet potato has three identical genomes (Shiotani and Kawase 1989), or that two genomes are identical and the other slightly different (Jones 1965; Ting and Kehr 1953). Thus, acceptance of Hypothesis I or II agrees well with the cytological evidence. The present results, due to the number of progenies used in our experiments, do not permit a further narrowing of the choice between Hypothesis I and II.

Based on Hypothesis I, it appears that the β-amylase null allele exists in high frequency in cultivated germplasm. Out of 14 clones (5 varieties and 6 advanced stocks from Japan, and 3 clones from other countries) used as normal parents, 3 clones were found to be simplex (β-Amy^1 β-amy^5), 5 to be duplex, 4 to be triplex, and the other 2 to be quadruplex, pentaplex, or hexaplex. Thus, the majority of the normal phenotypes is likely to be either simplex or duplex. An exact allele frequency cannot be estimated from the present data. However, it is clear that the β-*amy* allele was more frequent than the dominant β-*Amy* in the material tested. β-Amylase nulls have also been reported in other breeding stocks (Martin 1987). Even though the β-*amy* frequency is high, the null phenotype is not commonly found, due to the polysomic nature of the crop and the trait's recessiveness.

Implications for breeding

The present research has demonstrated that sweet potato can easily be bred for low β-amylase activity, first, because the trait is simply inherited, second, because the β-*amy* allele exists in high frequencies in cultivated material, and third, because a rapid screening method is available that can be applied in a practical breeding program. Even if the number of null genotypes in a population is not sufficient to maintain a wide genetic background, any clone of the null class can be used as a tester to quickly check the genotypes of any breeding material, and then the simplex or duplex genotypes can be selectively used to develop a low β-amylase population. Finally, the lack of β-amylase in storage roots does not appear to produce any negative effects. Satsumahikari has extremely low β-amylase activity but is high yielding, and no obvious negative side effects due to this trait have been reported (Kukimura et al. 1988). However, it is necessary to know which other factors are important for processing, and what type of genetic variability is available to make the sweet potato more suitable for processing. Similarly, non-sweetness is one of the qualities, although not the only one, that gives to a sweet potato a sufficiently agreeable taste to be used as a staple food.

The functional nature of the β-*amy* allele is not clear. If β-*Amy* is a structural gene, sweet potato has only one set of structural genes instead of a gene family as found in other crops (Kreis et al. 1988; Ainsworth et al. 1983), or several alleles are tightly linked and lost by a mutation effect (i.e., by deletion of a complex structural locus).

Alternatively, the null allele is a mutant of a regulatory locus affecting a family of structural genes coding for β-amylase activity. The Risø mutant 1508 of barley contains only trace amounts of β-amylase (Kreis et al. 1987) just as our β-*amy* mutant has. The barley mutant (*lys*3a) does not produce m-RNA of β-amylase, probably because the mutation affects the regulation of expression of β-amylase genes on other chromosomes. Another similarity between *lys*3a and β-*amy* is that they both have tissue-specific expression. The *lys*3a does not inhibit β-amylase production in other tissue (i.e., shoot and leaves): some sweet potato genotypes were found to have normal β-amylase activity in stems in spite of no β-amylase production in their storage roots (T. Kumagai, unpublished results).

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