



Clone x Tester Crosses on Compatibility Level of Sweetpotato (*Ipomoea batatas* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FCI, RI, YB and TS designed the study. Authors JR, WR and FCI performed the statistical analysis and wrote the protocol. Authors FCI, DH and MJM wrote the first draft of the manuscript. Authors TS and MJM managed the analyses of the study. Authors FCI, RS and DH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Evaluation of compatibility on sweetpotato is very important to determine the crossing parents to increase the compatibility of controlled cross-pollination. This research was aimed to study the level of compatibility in controlled cross-pollination based on percentage of normal seedlings obtained from crosses. The research was conducted at Kendalpayak Experimental Station of ILETRI, Malang, Indonesia then followed by breaking seed dormancy and germination of seeds obtained. The genetic materials used were 17 accessions with high yielding potential and 3 accessions as tester for crossing. The results showed that there were differences in the compatibility levels of crossing combination and their compatibilities as male or female parents. Clone C-011 (Tester 2) was the most compatible as male parent, while clone C-141, C-007 and C-131 were the most compatible as female parents. Clone C-127 (Tester 3) was the most compatible as both male and female parents. Clone C-001 (Tester 1) could not be used as female parent due to high incompatibility. This compatible clone information is very useful in determining of both controlled crosses and open crosses in sweet potato in order to increase the breeding efficiency.

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1. INTRODUCTION

New genetic diversity can be developed through controlled crosses and open crosses. Populations resulting from sweet potato (*Ipomoea batatas* L.) crossing among different clones will have high heterosigosity. Parents having the characteristics of self-incompatibility, cross-incompatibility and sterility lead to very low success of crosses. The flowering ability of each clone varies, even some clones were difficult to flower. The balance of the flowering ability of the parents of the crosses was highly required for open pollination (polycross) or hand pollination (controlled crosses)[1].

Sweet potato has chromosome $2n = 6x = 90$, have two types of chromosomes based on compatibility, namely: group A consist of species that were of the nature of species self-compatible (SC): *Ipomoea lacunose*, *Ipomoea triloba*, *Ipomoea trichocarpa*, *Ipomoea tiliaceae* and *Ipomoea gracilis*, grup B consist of *Ipomoea trifida*, *Ipomoea litoralis* and *Ipomoea batatas*[2]. Species in group B have a sporophytic SI type, a self-incompatible system that occurs when pollen falls on the pistil, but pollen does not occur so that fertilization does not occur and fertilization does not occur [3]. Meanwhile, if cross pollination and compatibility were carried out, pollen germination will occur 10-20 minutes after pollination [4].

Selfing (self-pollination on one plant) generally causes sterility, only a few clones were fertile [5]. There were two types of sterility in sweet potatoes, namely: (a). Sterility occurs in the pre-pollination phase, sterile pollen was found due to the disruption of the process of meiosis that produces gametes that were not viable and not functional, and (b) sterility occurs due to incompatibility in the process of fertilization which occurs in the post-pollination phase caused by pollen-tube was unable to penetrate the stigma, pollen-tube was unable to pass through the entire stylus, pollen-tube was not successful in the process of fertilization, ovules do not develop, seeds do not have endosperm, seeds do not germinate, seeds were weak and die, seeds do not grow into perfect plants and plants do not flower [6]. The presence of pollen sterility and incompatibility between pollen-stigma was the cause of the low capsule formation in sweet potatoes [7]. In the open pollination method, seeds were obtained by planting selected clones in the same area and crossing occurs naturally

with the help of wind and insects. The increase in genetic diversity in these methods was lower than in controlled crosses, besides it was inefficient for genetic studies because the source of pollen (male parent) was unknown [8].

Controlled crosses with many parents involved and performed reciprocally aimed at increasing heterosigosity in F_1 . High genetic diversity has the possibility of obtaining new individuals in the F_1 population. The Level of incompatibility in sweet potatoes depend on each combination of crosses. So far, the level of incompatibility was calculated based on the percentage of capsules/fruitsets. But in reality the capsules that was formed not all capsule contain of seeds, the percentage of empty seeds from crosses was also very high and seeds that contain embryos do not necessarily grow into normal seedlings. Therefore determining the level of incompatibility in sweet potatoes based on the percentage of normal seedlings will be more accurate [9]. Therefore information on the level of compatibility combination of clones, as male, female and both becomes important in determining the crossing parents. The objective of this study was to determine the level of compatibility of each combination of sweet potato crosses based on the percentage of normal seedlings.

2. MATERIALS AND METHODS

2.1 Study Site

Controlled crossing of sweetpotato clones was carried out in the Kendalpayak Experimental Garden, from March - October 2015 at Kendalpayak Research Station, Malang, East Java, Indonesia. Kendalpayak Research Station lies at $8^{\circ} 2' 56.4''$ LS $112^{\circ} 37'30''$ BT with an altitude of 445 m a.s.l. Furthermore, seed nurseries from the crossing were carried out in the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) greenhouse in Malang, East Java, Indonesia.

2.2 Planting Materials

The materials used were high-yielding potential clones of 17 clones and 3 clones as testers. The agricultural equipment used in the study were: petridish, crossing equipment. Crosses were performed reciprocally between clones that have high yield potential. The clones which have high yield potential, the amount of dry matter having a

rough 23.32-35.02%, and the ability to flower is moderate to very fertile. The characteristics of the clones are as described in Tables 1 and 2.

The selection of the crossing parents in sweet potato, besides the desired character also needs to consider the flowering ability and the degree of incompatibility. The choice of many flowering parents will increase the opportunity to enhance the number of crosses and the success of capsules and seed formation.

Each clone was planted in a plot measuring 1 m x 5 m with a spacing between 1 m ridges, a spacing between plants 25 cm and a spacing between ridges 30 cm, each plot consisted of 20 plants. Controlled crosses used the Basuki method [10] as follows: crosses were performed without emasculation (the crossed clones were known self-incompatible). Method of crossing: flower buds that would bloom the next day were first tied using a string or thread. This method was to avoid contamination of other pollen by insects and wind. Binding of flowers was done around 13:00 till finished by which the flower started to open for the next day at around 06:00 - 10:00 for flowers to open maximum and to make easier for us carrying out a crosses.

The steps of crossing sweet potato clones were as follows: (a). flowers to be made as male parents were picked and placed in plastic boxes. (b). female parent to be crossed were labeled using a color thread (the color of the thread matches the male parent to be crossed). (c). Then the staples of the male parent flower were opened. (d). pollen was taken using tweezers. (e). the ends of the female parent flowers were also untied. (f). pollen was then sprinkled on the surface of the stigma of the female parent. (g). the pollinated stigma was covered again with the flower crown and tied back with thread.

2.3 Sample Collection

Harvesting was done when the capsule/fruit set was dry and brown but has not broken. The harvested capsules were put in a labeled envelope. Furthermore, the capsules were dried under the sun rise for about three days. The dried capsules were then stored in a paper bag, labeled with a cross code and placed in the seed storage room. Observations made at this cross include:

- a). The number of flowers that was crossed and the number of fruit sets formed;

- b). Percentage of fruit set, i.e. the number of fruit sets formed at each cross combination based on Indriani [9].

$$\% \text{ of fruit set} = (N_f / \sum N_{pf}) \times 100\%$$

Where: N_f = number of fruit sets; $\sum N_{pf}$ = total numbers of pollinated flowers

- c). The average number of seeds per fruit set produced by each cross combination.

$$\text{Average number of seeds} = N_s / N_f$$

Where: N_s = number of seed; N_f = number of fruit sets.

The calculation was done after the seeds were cut off the hilum tip, if the tip of the radicles appeared, it mean that there was an embryo. While seeds that do not contain embryos will look empty. Each cross combination was counted by the number of seeds containing the embryo and the empty ones.

- d). Percentage of normal seedlings

The seeds containing embryos were then planted on polybags with a diameter of 3 cm, the percentage of germination was calculated on the seventh day with the following formula:

$$\% \text{ of normal seedlings} = (N_{ns} / \sum N_{st}) \times 100\%$$

Where: N_{ns} = number of normal seedlings; $\sum N_{st}$ = total number of seeds tested.

2.4 Data Analysis

Determination of the incompatibility level based on the percentage of normal seedlings was as follows: compatible if the normal seedlings account for more than 20%, partially compatible if the normal seedlings account between 10-20%, very incompatible if the normal seedlings account less than 10%, fully incompatible 0%, if no normal seedlings is produced [9].

3. RESULTS AND DISCUSSION

Compatibility level based on % normal seedlings of each crossing parents and its reciprocity with clone is shown in Table 3. Crosses amongs 17 clones with high yielding potential and 3 tester clones, resulted as many as 5,545 crosses, 1,296 formed capsules or fruit sets (23.4%), 1956 seeds (35.3%), 1,101 embryo-shaped

seeds (19.9%) and 530 growth to normal seedlings (9.6%). The total number of crosses and the percentage of success in obtaining new clones was 9.6% Table 3.

The low percentage of normal seedlings produced was caused by many factors that influence. Physically, reproduction in sweetpotato is blocked because of several things including: flowering in sweetpotato is influenced by photoperiodisme, the receptive period is quite short and there is incompatibility [11]. Flower production is genetically controlled and influenced by the environment, several techniques used to stimulate flowering include temperature, photoperiodisme, manipulation of nutrition and the use of hormones [12].

The failure to produce new clones caused a sterility before pollination. The presence of sterile pollen can disturb the meiosis, while incompatibility during pollination causes pollen tubes to be unable to penetrate the stigma, ovules do not develop and the formation of seed structures is imperfect. Incompatibility during the pollination process until fertilization results in seed without embryos (empty seed). This causes the normal seedlings produced very low [6]. Therefore, information on the combination of compatible clones, as male, female and both becomes important in determining the crossing parents. The level of incompatibility depends on each combination of crosses.

3.1 Number of Crosses

The crosses number of 17 high yielding Clones with three Testers of each crossing parents

(Clone as female x Tester as male) and its reciprocal (Clone as male x Tester as female) is presented in Table 4. When Clone used as female, the mean of crosses number of 17 clones with Tester 1, Tester 2, and Tester 3 were 71.5, 71.0, and 71.8, respectively, which was not difference among Testers. However, when Clone used as male, the mean of crosses number of 17 clones with Tester 1, Tester 2, and Tester 3 were 24.5, 31.8, and 48.8, respectively which was difference among Testers Table 4 and Fig. 1.

3.2 Number of Normal Seedling

The number of normal seedling derived from crosses of 17 high yielding potential clones with three testers of each crossing parents (Clone as female x Tester as male) and its reciprocal (Clone as male x Tester as female) is presented in Table 5. When Clone used as female, the mean of normal seedling derived from crosses of 17 clones with Tester 1, Tester 2, and Tester 3 were 17.92, 41.81, and 27.81, respectively, which was difference among Testers. However, when Clone used as male, the mean of normal seedling derived from crosses of 17 clones with Tester 1, Tester 2, and Tester 3 were 0, 18.5, and 28.86, respectively which was difference among Testers Table 5 and Fig. 2. Clone C-141 gave the highest of number of normal seedling among 17 clones of 51.7, 58.2, and 43.9 with Tester 1, Tester 2, and Tester 3, respectively. However, when Clone used as male or reciprocal crosses, the mean of crosses number of 17 clones with Tester 1, Tester 2, and Tester 3 were 0, 76.5, and 33.3, respectively Table 5.

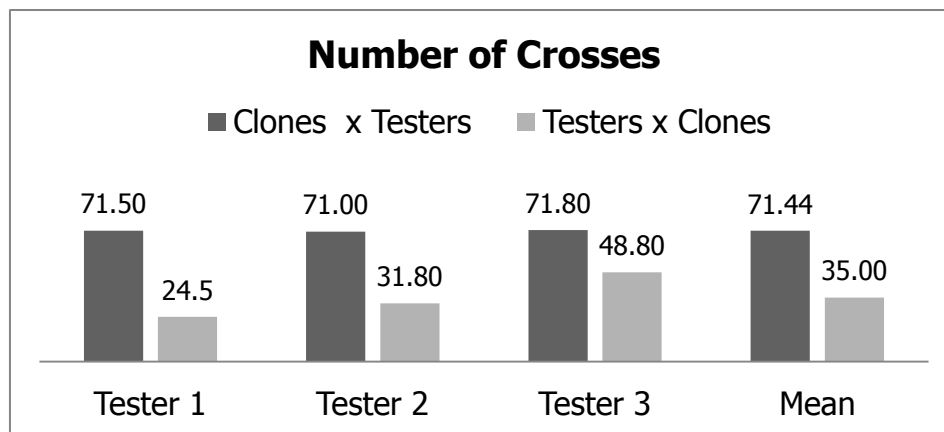


Fig. 1. Crosses number average of 17 high yielding potential clones with three testers of each crossing parents (Clones x Testers¹⁾ and its reciprocal (Testers¹⁾ x Clones)

¹⁾Tester 1 = C-001 (Cangkuang); Tester 2 = C-011; and Tester 3 = C-127

Table 1. List of the characteristics of sweet potato clones with high yield potential

No.	Clone code	Clone Name	Yield Potential (ton/ha)	Dry matter (%)	Flowering ability	Tuber Color ¹⁾	
						Skin	Flesh
1	C-139	MSU 07023-86	50.83	23.32	high	R5	O4
2	C-053	UJ-35 Batatas Merah	48.85	25.41	high	Y5	Y4O1
3	C-083	MSU 07031-28	45.11	24.08	low	R2	O3
4	C-145	MSU 07022-15	42.92	26.65	intermediate	Y3	Y3
5	C-125	MSU 07022-12	37.02	30.34	high	Y2	Y2
6	C-141	MSU 07015-54	30.03	33.90	very high	R4	W
7	C-062	MSU 10039-03	39.96	31.07	high	Y2	Y1
8	C-039	UJ-16 Slape	36.34	25.79	high	O1	O4
9	C-007	Papua Solossa	31.94	34.25	very high	Y3	Y3
10	C-004	Kidal	31.58	35.02	high	R4	Y4
11	C-026	Cilembu-1	30.64	30.38	intermediate	Y4	Y3O3
12	C-016	IR Melati	33.80	24.19	intermediate	R4	Y1
13	C-117	RWAS 10062-01	32.45	29.70	high	P5	P5
14	C-065	MSU 10048-09	33.17	26.81	high	Y2	W
15	C-131	MSU 07012-06	36.29	25.38	high	R4	O3
16	C-090	MSU 09008-92	37.54	29.77	high	R6	P5
17	C-123	MSU 07009-75	32.18	32.98	high	R4	Y2

¹⁾ Skin color and tuber flesh: red (R), white (W), yellow (Y), orange (O) and purple (P);
Color intensity consists of: 7 = very dark, 6 = dark, 5 = slightly dark, 4 = bright, 3 = pale, 2 = slightly pale and 1 = very pale

Table 2. List of the characteristics of sweet potato clones used as testers

Tester	Clone code	Clone Name	Yield Potential (ton/ha)	Dry matter (%)	Flowering ability	Tuber Color ¹⁾	
						Skin	Flesh
T1	C-001	Cangkuang	30.00	35.2	high	R4	Y2
T2	C-011	Beta-2	36.81	23.7	high	R6	O3
T3	C-127	RIS 10068-02	38.86	31.1	high	Y5	Y3O3

¹⁾ Skin color and tuber flesh consist of: red (R), white (W), yellow (Y), orange (O), and purple (P).
Color intensity consists of: 7 = very dark, 6 = dark, 5 = slightly dark, 4 = bright, 3 = pale, 2 = slightly pale and 1 = very pale

Table 3. Number of fruit set, seed, and normal seedling obtained from crosses of 17 high yielding potential clones with three testers

	Crosses	Fruit set	Total Seed	Filled seed	Normal seedlings
Number	5,545	1,296	1,956	1,101	530
Percentage (%)	100.0	23.4	35.3	19.9	9.6

Table 4. Crosses number of 17 high yielding potential clones with three testers of each crossing parents and its reciprocal

No.	Clone code	Number of crosses							
		Clone x Tester ¹⁾				Reciprocal (Tester x Clone) ¹⁾			
		Tester 1 ²⁾	Tester 2 ²⁾	Tester 3 ²⁾	Mean	Tester 1 ²⁾	Tester 2 ²⁾	Tester 3 ²⁾	Mean
1	C-139	164	134	97	131.67	17	54	61	44.0
2	C-053	78	74	55	69.00	7	23	46	25.3
3	C-083	25	16	32	24.33	41	31	59	43.7
4	C-145	79	72	74	75.00	15	34	68	39.0
5	C-125	79	48	52	59.67	61	76	73	70.0
6	C-141	146	145	219	170.00	38	37	26	33.7
7	C-062	34	61	68	54.33	16	8	51	25.0
8	C-039	82	123	174	126.33	21	72	96	63.0
9	C-007	74	51	91	72.00	15	31	48	31.3
10	C-004	73	43	35	50.33	35	65	57	52.3
11	C-026	102	117	92	103.67	58	31	67	52.0
12	C-016	65	68	79	70.67	11	4	39	18.0
13	C-117	61	59	62	60.67	56	16	43	38.3
14	C-065	45	51	58	51.33	8	28	46	27.3
15	C-131	50	50	65	55.00	18	20	15	17.7
16	C-090	13	50	25	29.33	0	0	22	7.3
17	C-123	46	45	11	34.00	0	10	59	23.0
Mean		71.5	71.0	71.8	71.44	24.5	31.8	48.8	35.0

¹⁾Clone x Tester = Clone (as Female) x Tester (as Male); ¹⁾Reciprocal = Tester (as Female) x Clone (as Male). ²⁾Tester 1 = C-001 (Cangkuang); Tester 2 = C-011; and Tester 3 = C-127

3.3 Level of Cross Compatibility

The level of cross compatibility derived from crosses of 17 high yielding potential clones with three testers of each crossing parents (Clone as female x Tester as male) and its reciprocal (Clone as male x Tester as female) is presented in Table 6. When Clone used as female, the mean of normal seedling derived from crosses of 17 clones with three Testers, namely Tester 1, Tester 2, and Tester 3 were 6, 13, and 9, respectively, which was difference among Testers. However, when Clone used as male, the mean of normal seedling derived from crosses of 17 clones with three Testers, namely Tester 1, Tester 2, and Tester 3 were 0, 6, and 9, respectively which was difference among Testers Table 5 and Fig. 3. Three clones (C-141, C-007, and C-131) gave the best level of cross compatibility of when crossed to three Testers (Tester 1, Tester 2, and Tester 3). However, when Clone used as male or reciprocal crosses, the mean of crosses number of 17 clones with three Testers, the level of cross compatibility only with Tester 2 and Tester 3 Table 6. These data suggested that the rule of clone used as female or male determined the the level of cross compatibility of crosses derived from crosses or there was a reciprocal difference in the number of the level of cross compatibility of sweet potato.

3.4 The Success Rate of Hand Pollination

The reciprocal crossing between 17 clones of high yield potential and Tester1 (C-001=

Canguang Variety) performed 1,633 crosses forming 15.3% capsules. Normal plants produced as much as 2.6%. Whereas the same clones crossed with Beta-2 varieties (C-011) performed 1,747 crosses produce 432 fertile capsules (24.7%) and normal seedlings that could be produced were 200 clones (11.4%). Whereas crosses with the number of C-127 as tester (Tester 3) were 2,165 times, able to form capsules as much as 28.4% and normal plants 288 (13.3%). The level of success in obtaining new clones were different among the parents. The number of crosses made was between 219-1,321 times. Each clone as female and male parents performed a number of 0 - 142 new clones Table 7.

The success of crossing each clone of all crosses performed, both male and female is listed in Table 7. Three clones used as testers have the ability to flower. Controlled crosses between Testers and 17 high yielding potential clones carried out with the reciprocal, have different success rates for crosses as shown in Table 7. Success of crosses based on percentage of capsules is between 13.2 - 40.3%. Whereas based on the percentage of normal seedlings, it shows the target 0 - 18.0%. In the test clones, it turns out that clone No. 1 is unable to produce normal plants when used as females. Clone C-001 and C-127 are more suitable as male parents. Clone 11 can be used as male and female parents. The success rate of crossing as male and female parents of both clones in producing normal seedlings is relatively balanced.

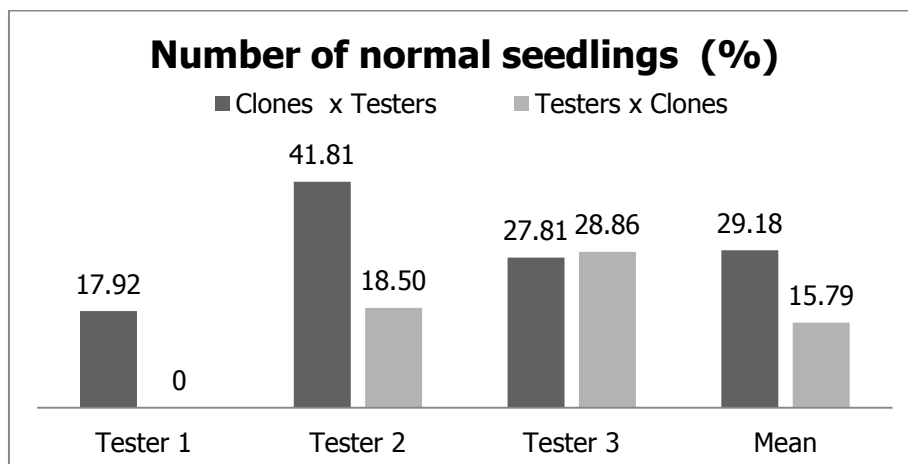


Fig. 2. Number of normal seedling average of crosses of 17 high yielding potential clones with three testers of each crossing parents (Clones x Testers) and its reciprocal (Testers x Clones)

¹⁾ Tester 1 = C-001 (Canguang); Tester 2 = C-011; and Tester 3 = C-127

Table 5. Number of normal seedling derived from crossing of 17 high yielding potential clones with three testers of each crossing parents and its reciprocal

No.	Clone code	Number of normal seedlings (%)							
		Clone x Tester ¹⁾				Reciprocal (Tester x Clone) ¹⁾			
		Tester 1 ²⁾	Tester 2 ²⁾	Tester 3 ²⁾	Mean	Tester 1 ²⁾	Tester 2 ²⁾	Tester 3 ²⁾	Mean
1	C-139	18.8	0	8.3	9.03	0	0	61.3	20.43
2	C-053	0	57.7	21.4	26.37	0	0	56.0	18.67
3	C-083	0	36.4	52.9	29.77	0	0	51.5	17.17
4	C-145	0	0	50.0	16.67	0	17.6	43.6	20.40
5	C-125	0	30.8	0	10.27	0	55.6	0	18.53
6	C-141	51.7	58.2	43.9	51.27	0	76.5	33.3	36.60
7	C-062	0	27.3	17.6	14.97	0	0	0	0.00
8	C-039	0	57.1	23.5	26.87	0	31.3	54.3	28.53
9	C-007	43.8	85.7	55.6	61.70	0	25.0	36.8	20.60
10	C-004	28.6	72.7	10.0	37.10	0	12.5	17.9	10.13
11	C-026	0	68.0	62.9	43.63	0	0	71.1	23.70
12	C-016	0	38.5	0	12.83	0	0	0	0.00
13	C-117	41.7	0	61.1	34.27	0	0	0	0.00
14	C-065	0	38.5	15,6	18.03	0	64.7	64.9	43.20
15	C-131	100	58.3	50.0	69.43	0	31.3	0	10.43
16	C-090	0	0	0	0.00	0	0	0	0.00
17	C-123	20	81.5	0	33.83	0	0	0	0.00
Mean		17.92	41.81	27.81	29.18	0	18.5	28.86	15.79

¹⁾ Clone x Tester = Clone (as Female) x Tester (as Male); ¹⁾ Reciprocal = Tester (as Female) x Clone (as Male). ²⁾ Tester 1 = C-001 (Cangkuang); Tester 2 = C-011; and Tester 3 = C-127

Table 6. Level of compatibility base on normal seedling derived from crossing of 17 high yielding potential clones with three testers of each crossing parents and its reciprocity

No.	Clone code	Level of Compatibility ¹⁾							
		Clone x Tester ²⁾				Reciprocal (Tester x Clone) ²⁾			
		Tester 1 ³⁾	Tester 2 ³⁾	Tester 3 ³⁾	Number of compatible ⁴⁾	Tester 1 ³⁾	Tester 2 ³⁾	Tester 3 ³⁾	Number of compatible ⁴⁾
1	C-139	PC	FI	VI	0	FI	FI	C	1
2	C-053	FI	C	C	2	FI	FI	C	1
3	C-083	FI	C	C	2	FI	FI	C	1
4	C-145	FI	FI	C	1	FI	PC	C	1
5	C-125	FI	C	FI	1	FI	C	FI	1
6	C-141	C	C	C	3	FI	C	C	2
7	C-062	FI	C	PC	1	FI	FI	FI	0
8	C-039	FI	C	C	2	FI	C	C	2
9	C-007	C	C	C	3	FI	C	C	2
10	C-004	C	C	PC	2	FI	PI	PC	0
11	C-026	FI	C	C	2	FI	FI	C	1
12	C-016	FI	C	FI	1	FI	FI	FI	0
13	C-117	C	FI	C	2	FI	FI	FI	0
14	C-065	FI	C	PC	1	FI	C	C	2
15	C-131	C	C	C	3	FI	C	FI	1
16	C-090	FI	FI	FI	0	0	0	FI	0
17	C-123	C	C	FI	2	0	FI	FI	0
Total number of compatible		6	13	9	28	0	6	9	15

¹⁾ C = compatible; PC = partial compatible; VI = very incompatible; FI = fully incompatible. ²⁾ Clone x Tester = Clone (as Female) x Tester (as Male); ²⁾ Reciprocal = Tester (as Female) x Clone (as Male). ³⁾ Tester 1 = C-001 (Cangkuang); Tester 2 = C-011; and Tester 3 = C-127, ⁴⁾ Number of compatible = number of "C" level in cross of each Clone with each Tester

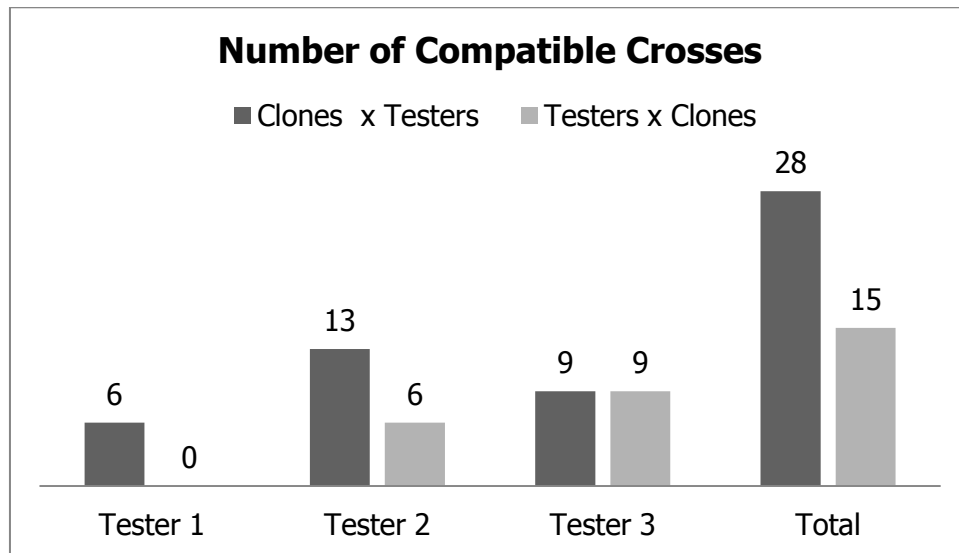


Fig. 3. Number of compatible level of crosses average of crosses of 17 high yielding potential clones with three testers of each crossing parents (Clones x Testers¹⁾ and its reciprocal (Testers¹ x Clones)

¹⁾ Tester 1 = C-001 (Cangkuang); Tester 2 = C-011; and Tester 3 = C-127

Based on the percentage of normal seedlings produced, the highest success was clone C-123 as females that was 18%. However, clone C-123 as males showed 0% of success (not suitable as males). The ability to produce normal seedlings that reach $\geq 10\%$ as a male include clone C-145, C-039, C-026 and C-065; while as a female include: clone C-141, C-007, C-026, C-131 and C-123 Table 8. Clone C-007 (Papua Solossa) was more suitable as a female parent with a compatibility level of 87.5% and a successful crossing of 13.7%. This may be caused by a high level of stigma receptivity pollen. Susanto et al. [13] reported that the stigma receptivity of the Papua Solossa variety showed 100%, which means that the clone was more suitable as a female.

Pollen sterility and the incompatibility of pollen-stigma interactions also play a role in causing low capsules formation [7]. Even in full incompatible combinations cannot produce embryonic-shaped seeds that can grow into normal plants. The compatibility of a cross is determined by two criteria, namely the failure or success of pollen germination in style and the success or failure of fertilization after pollination [14].

Low percentage of normal seedlings produced was due to many factors that influence. Physiologically, reproduction is blocked due to flowering in sweetpotato is influenced by

photoperiodism, the receptive period is quite short and there is incompatibility [11]. Flower production is genetically controlled and influenced by environment, several techniques used to stimulate flowering include temperature, photoperiodism, manipulation of nutrition and the use of hormones [12].

Capsules of the physiologis cross-ripening about one month after crossing. Characteristics of capsules that are ready to be harvested are marked by the color of the capsules which is brown/black and dry, before breaking it must be harvested immediately. Capsules usually have 1-3 seeds depending on the combination of the crosses and the degree of incompatibility. Seed coat is very hard, its viability can last for a long time. At 50% humidity and a temperature of 18 °C, viability can be maintained for 20 years [15]. Seed weight of 1.3 - 3.0 g per 100 seeds depends on a combination of crosses [16]. Hard sweet potato seed skin requires special treatment for the break of its dormitory, using the hilum cutting method [17].

The embryonic and empty seeds will be seen after hilum cutting, then the viability of the embryonic seeds were tested. The success of fertilization can be seen from the capsules formed, in a compatible crossbreed combination, generally capsules with 1 or 2 seeds sometimes have 3 seeds. Gruneberg et al.[1] states that in controlled crosses can be obtained 1-3 seeds per

Table 7. Percentage of successful crosses of high yielding potential clones with three clone testers

No.	Clones code	Parent as	Number of crosses	Number of capsules	Fertile capsules (%)	Number of seeds	Number of fertilized seeds	New clones	Success of crosses (%)
1	C-139	Female	1,068	148	13.9	179	121	25	2.3
		Male	509	157	30.8	210	145	44	8.6
2	C-053	Female	532	129	24.2	166	94	36	6.7
		Male	404	133	32.9	158	50	28	6.9
3	C-083	Female	278	112	40.3	151	62	19	6.8
		Male	364	86	23.6	112	33	17	4.7
4	C-145	Female	556	49	8.8	55	20	7	1.3
		Male	411	160	38.9	251	144	56	13.6
5	C-125	Female	456	70	15.4	91	34	8	1.8
		Male	572	88	15.4	109	64	30	5.2
6	C-141	Female	1,321	320	24.2	486	292	142	10.7
		Male	421	209	49.6	355	107	42	9.9
7	C-062	Female	449	50	11.1	86	56	19	4.2
		Male	408	110	26.9	145	49	11	2.7
8	C-039	Female	996	93	9.3	94	44	23	2.3
		Male	565	194	34.3	298	210	89	15.8
9	C-007	Female	593	196	33.1	332	146	81	13.7
		Male	374	120	32.1	166	48	20	5.3
10	C-004	Female	509	74	14.5	101	43	14	2.8
		Male	526	164	31.2	225	112	26	4.9
11	C-026	Female	719	167	23.2	225	124	79	10.9
		Male	431	147	34.1	218	85	44	10.2
12	C-016	Female	577	78	13.5	110	42	25	4.3
		Male	303	35	11.6	29	8	6	1.9
13	C-117	Female	499	104	20.8	130	97	43	8.6
		Male	413	93	22.5	111	38	1	0.2
14	C-065	Female	445	86	19.3	130	63	15	3.4
		Male	279	95	34.0	145	72	40	14.3
15	C-131	Female	460	121	26.3	154	136	77	16.7

No.	Clones code	Parent as	Number of crosses	Number of capsules	Fertile capsules (%)	Number of seeds	Number of fertilized seeds	New clones	Success of crosses (%)
16	C-090	Male	376	172	45.7	228	88	16	4.3
		Female	252	39	15.5	41	27	12	4.8
17	C-123	Male	219	57	26.0	69	24	5	2.3
		Female	311	101	32.5	127	94	56	18.0
		Male	260	5	1.9	6	0	0	0

capsules. The results showed a high percentage of empty seeds, and the number of embryonic seeds did not grow into normal plants.

Cross incompatibility can occur on the surface of stigma due to incompatible between the pollen substance and the exudate on the stigma. Whereas sterility can be caused by pollen tube failure from stigma to penetrate the force toward the ovary [18]. Sterility in autopolyploid plants causes chromosomal dual distribution to meiosis not normal [19]. Sterility, incompatibility and environmental factors affect various stages of the reproductive process. These factors cause germination and seed vigor to be low, abnormal plant types, reduced flowering, ovul and embryo abortion and low seed formation [15]. The low number of seeds formed causes the percentage of normal plants produced is also low. This can cause failure in fertilization, according to [16], there are two causes, namely pollen germination, but fertilization fails and the embryo grows weak and even dies after fertilization.

Evaluation of the level of incompatibility of each cross combination is needed to obtain information on the suitability of each clone as male, female and both. High compatibility between the parents is expected to form normal capsules, seeds and seedlings that have the desired characteristics. According to Martin[6], differences in the incompatibility level of each combination may cause failure in the following stages: the stage before pollination due to male and female gametes are not normal. Pollination stage is generally caused by pollen that does not germinate and stigma does not stimulate germination. The stage after pollination often results in the pollen tube being unable to penetrate the stigma and pistil stems so that it does not reach the ovule. Other factors affect include ovules that are not fully developed and abnormality of endosperm, resulting that the seeds do not germinate, seedlings grow weak, abnormal and even die.

Conventional sweet potato breeding through hybridization is an effective way in obtaining superior sweet potato clones. The existence of self-incompatibility (self incompatible) and cross incompatible (cross incompatible) is an obstacle to hybridizationsweet potato [20]. Basically, incompatibility system in *Convolvulaceae* family (for example sweetpotato), Brassicaseae, and Compositae are sporophytic, meaning incompatibility is determined by the phenotype of the pollen not the gametes [21]. The factors

affecting the failure of capsules/seed formation in controlled crosses also causes sterility after pollen germination. However, there is a failure in the formation of pollen tubes resulting that fertilization does not occur, embryo abortion occurs or seedlings grow abnormally/weakly [16]. Meanwhile, according to Martin [6] sterility occurs in the pre-pollination phase, due to the presence of sterile pollen (not viable) and in the post-pollination phase that occurs during the fertilization/fertilization process. The availability of parents with cross-incompatibility cause to very low success of crosses [22].

The process of fruit and seed formations in a controlled cross-pollination is a complex mechanism because of the cross-incompatibility issue which appears in sweetpotato. Seed vigor produced depends on each combination cross compatibility. The quality of one seed lot was reflected on the seed vigor counted based on the on-site growth rate [23]. Incompatibility may occur on the surface of stigma due to a mismatch between the pollen substance and the exudate on the stigma. Whereas sterility may cause pollen tube failure from stigma to penetrate the force toward the ovary [18]. Sterility in autopolyploid plants causes chromosomal dual distribution to meiosis not normal [19]. Sterility, incompatibility and environmental factors affect various stages of the reproductive process. These factors cause germination and seed vigor to be low, abnormal plant types, reduced flowering, ovul and embryo abortion and low seed formation [15]. The low number of seeds formed causes the percentage of normal plants produced is also low. Failure in fertilization are caused by pollen germination but fertilization fails and the embryo grows weak even dies after fertilization[16].

Fertilization of the embryo grows is very important in developing high yielding sweet potato variety since we need a cheap price of carbohydrate source for human consumption from the sweet potato tuber yield as well as for animal feed from sweet potato leaves. The possibility of converting sweet potato leaves to animal feed through fermentation is encouraging technology [24].

4. CONCLUSION

The Level of compatibility of each cross combination showed differences, by which each parent showed its suitability as male parent and female parent. Clone C-011 (Tester 2) was the

most compatible as male parent, while clone C-141, C-007 and C-131 were most compatible as female parents. Clone C-127 (Tester 3) was the most compatible as both male and female parents. Clone C-001 (Tester 1) could not be used as female parent due to high incompatibility. This compatible clone information is very useful in determining of both controlled crosses and open crosses in sweet potatoes in order to increase breeding efficiency.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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