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Normal Seedlings as A New Parameter for Predicting Cross-Incompatibility Level on Sweetpotato

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ABSTRACT

This research aimed to investigate the incompatibility levels of controlled cross-pollination in sweetpotato, based on normal seedling percentage and their correlation with seed vigour. The controlled cross-pollination in sweetpotato faces issues due to its cross-incompatibility and self-incompatibility characteristics. Currently, the incompatibility level in sweetpotato is investigated based on the fruit set percentage. However, this criterion lacks the ability in accurately predicting the number of new clones. Therefore, it is essential to study new parameters to create a better investigation of incompatibility in sweetpotato. The materials used in this research consisted of eight sweetpotato clones as female and four sweetpotato clones as male parents. Cross-pollinations were done reciprocally. The experiment was conducted at Indonesian Legume and Tuber Crops Research Institute from April to December 2014. The result showed that totally 5,188 times crossing produced about 25% fruit sets and 10% normal seedlings. The use of normal seedlings percentage as a new parameter in evaluating cross-pollination has apparently seemed to be more effective than the fruit sets percentage method because numbers of new clones could be known accurately. This research revealed that the normal seedling could be used as a new parameter in determining the incompatibility level in sweetpotato controlled cross-pollination.

Keywords: incompatibility; normal seedlings; seed vigour; sweetpotato

INTRODUCTION

The high nutrition and production of sweetpotato are known as great potentials to develop this commodity as a raw material for the healthy food industry. Currently, the uses sweetpotato food products have become varied, thus gaining tremendous popularity. Therefore, the development of sweetpotato as a commodity is essential for the national food diversification policy. Nevertheless, variation improvement efforts, as an important adequate technology, are not to be neglected (Zuraida & Supriati, 2001).

Sweetpotato reproduction aims to produce seed, but for production of the crop is propagated vegetatively from stems, young shoots produced by the roots, and rooted leaves (Martin & Jones, 1986). The problems in sweetpotato plant breeding programs such as flowering ability, incompatibility and sterility cause poor fruiting and seed setting (Wang, 1975). Pod and seed production can be increased by treatment of flowering, open pollinated plants with suitable insecticides (Martin & Jones, 1986).

The identification of incompatibility has not eliminated the problem of low percentage fruit set and seeds (Wang, 1968). Main factor that cause limited ability to produce true seed is a system of self-and cross-incompatibility (Martin, 1965) and other factors such as; environment, genetics, morphological, pathological, and physiological factors contributing their influences (Wang, 1968). Meanwhile, the hexaploid nature of the species also contribute to low fertility. So these factors are expressed at various stages as low seed germination, abnormal seedlings increases, flowering reduction, ovule abortion, ineffective pollen tube growth or embryo abortion, and poor seed set (Jones, 1980).

Sweetpotato cytogenetically is classified as autopolyploid with a hexaploid type (6n) and 90 numbers of chromosomes (Martin, 1981). This crop is an autopolyploid and highly heterozygous clone hybrid (Grüneberg et al., 2015). Conventional breeding on sweetpotato through cross-pollination

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is still effective in providing clones that create new genetic variety. However, this process entails self and cross-incompatibility issues (Gasura, Mashingaidze, & Mukasa, 2008). Beside the information on flower ability in controlling cross-pollinations, incompatibility levels of the desired parents are also important. On the other hand, in open cross-pollination, the selected parents are grown closely to allow cross-pollination among these parents, and the seeds formed by this method are then used as a basic material of selection. So, it cannot be identified the incompatibility among the parents.

In cross incompatibility cases, the dominating gene role is the most determining factor. Meanwhile, the sweetpotato clones will be incompatible with other clones which have a similar genetic structure (Gurmu, Hussein, & Laing, 2013). The self-incompatibility in sweetpotato is sporophytic and controlled by multiple genes (Kowyama, Shimano, & Kawase, 1980). Wheeler, Franklin-Tong, & Franklin (2001) found that the self-incompatibility (SI) gene controls self-incompatibility in sweetpotato. This self-incompatibility system do not linked to other traits, it is identifiable from pollen germination failure after the pollination occurs. Thus, fertilization will not occur neither any seed is formed. Meanwhile in cross-incompatibility, the pollen will germinate on the stigma surface 10-20 minutes after pollination (Kowyama, Tsuchiya, & Kakeda, 2000).

In controlled cross-pollination, incompatibility causes the low percentage of fruit set and seed produced, not all the formed seeds are filled with an embryo. Moreover, in full incompatibility cases, the seeds even cannot germinate. Martin & Cabanillas (1966) reported that two types of barriers can be described, the first is pollen germination that occurs but fails fertilization and the second is weakness or death of the embryo that occurs after fertilization. Furthermore, the process of embryo or endosperm development causes in aborted scales, small and malformed seeds, empty seeds, not germinating seed and kill young seedling. Lestari (2010) finds that the low fruit formation rate and seed in sweetpotato are caused by incompatibility and sterility aspects. Meanwhile, the controlled cross-pollination shows some abnormal ovaries which might be an indication of some irregularity in ovule ontogeny which caused the ovules incapable of being fertilized and ultimately fruit set and seed formation (Wang, 1968).

Wang (1964) argues that the determination of incompatibility level of a cross combination can be classified as compatible if it produces fruit set more than 20 %; partially compatible to 10-20 %; very incompatible less than 10 %; and fully incompatible if no fruit set is produced. 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. Filho (2015) stated that the quality of one seed lot was reflected on the seed vigor counted based on the on-site growth rate. The utilization of seed vigor as a physiological marker to identify the quality of one seed lot is apparently better than any antioxidant enzymes found in any common bean (*Phaseolus vulgaris* L.) (Moriya, Neto, Marks, & Custódio, 2015).

Generally, the determination of incompatibility in a controlled cross-pollination is general inadequate until its ability to produce fruit and seeds. This is due to the formed fruit does not contain any seed yet, and the formed seed does not always contain an embryo. On the other hand, the seed with an embryo is not always able to grow as normal seedlings. Therefore, it is crucial to identify new parameters which can be used as a basis to determine the level of incompatibility as well as to predict the number of new clones expected to be obtained. The normal germination percentage of the seeds with embryo is known after seven days. This is also useful to predict the numbers of new clones expected in a more accurate manner. The classification of incompatibility level based on normal seedlings is proposed as follows; compatible, if the normal seedlings account for more than 20 %, partially compatible 10-20 %, very incompatible less than 10 %, and fully incompatible if no normal seedlings is produced. This research aimed to evaluate the incompatibility level based on the percentage of normal seedlings and its correlation to vigor seeds as products of controlled cross-pollination in sweetpotato.

MATERIALS AND METHODS

The research was conducted from April to December 2014 at Indonesian Legumes and Tuber Crops Research Institute. Materials used in this experiment were eight sweetpotato female parents namely Kidal (A), Papua Solossa (B), IR.

Melati (C), Cilembu-1 (D), UJ-16 Slape (E), MSU 07022-12 (F), MSU 07023-86 (G), MSU 07015-54 (H). Meanwhile, male parents consist of four clones namely Beta-2 (I), MSU 07022-14 (J), MSU 07009-12 (K) and RIS 10068-02 (L). The number of pollination combination with their reciprocal were 64.

Pollination Method

The parent clones were chosen from an identification process of germplasm in 2013 and planted in a 1 m x 5 m plot with an average spacing of 25 cm. The distance between the peaks was 1 m and 30 cm between ridges. Each block consisting of 20 plots and each plot contained 20 plants. Induction of flowering was done especially for clones with low flowering ability by spraying Gandasil B per 10 days for flowering period at a concentration 15g $10L^{-1}$. Crossing was done on clones with the same flowering time. The crossing process took a long time (7 months), because clones with late flowering time were also crossed.

The cross-pollination employed the method introduced by Basuki (1986) without any emasculation. The clones were 100 % self-incompatible. The steps could be described as follows: Step 1, the flowers which ready to bloom at the next day were tied to prevent contamination from other pollen (done at around 1-4 p.m.), as seen in Figure 1 and a flower was ready for pollinating next day in Figure 2.

Step 2, the male parent, flowers were picked and placed in plastic boxes. Step 3, the female parent, flowers was labeled on their stems. Step 4, the male parent flowers were then untied to obtain the pollen using tweezers then were put on the stigma's female. Step 5, the pollinated flowers then were tied again to prevent contamination from other pollen. The crossing was done from 6.00-10.00 a.m. using the same method as the reciprocal crossing.

Harvesting and Processing of the Seed

The harvesting and seed processing was done as follow; the brownish and dried fruits were picked before cracking as seen in Figure 3. They were then put into an envelope and dried under the sun shine, after the fruit was dried, than fruit coat was removed to obtain the seed, and similarly, put into an envelope and dried for 1-3 days under the sun shine, Once the seeds were totally dried, they were stored in a labeled paper bag and kept into seed storage at 10 ºC.

Figure 1. Flower was tied, one day before bloom

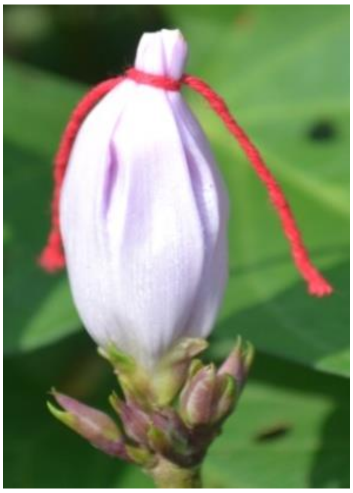

Figure 2. Flower was ready for pollinating

Figure 3. Mature fruit set

Figure 4. Performance of seedlings before transplanting to field in compatible combination 26 x 127 (D x L) and their reciprocal crossings 127 x 26 (L x D) based on normal seedlings (3 weeks after planting)

Dormancy Breaking and Seed Testing

The thick nature of sweetpotato seed coat blocks the imbibition processes that leads the seeds to a dormant condition. Jayasuriya, Baskin, Geneve, & Baskin (2007) used a physical technique seed by cutting the hilum tip of *Ipomoea lacunose*. The dormancy breaking in sweetpotato was also done using a similar method. The black hilum tip (F1) was cut to identify whether it contained an embryo. Seeds with embryos were then planted in a single pot with a diameter of 3 cm. Seven days after planting, they were observed on number of normal seedlings and number of strong normal seedlings. After three weeks, the normal seedlings were then transplanted to the field, as seen in Figure 4.

Observation

Some parameters were investigated, i.e.; the number of flowers being crossed, the number of fruits, seeds, seeds with an embryo, and normal seedlings. Percentages of fruit sets using the following formulation:

$$\% \text{ of fruitset} = \frac{\text{Nf}}{\sum \text{Npf}} \times 100 \%$$

Where:
Nf = number of fruitsets
∑ Npf = total numbers of pollinated flowers

Seed vigour according to its seed germination was determined by calculating the number of strong normal seedlings (seedling growth normally without damage in any part) on seven days after planting, using the following formulation by Sadjad (1994):

$$\% \text{ of simultaneously growth} = \frac{\text{Nsns}}{\text{Nst}} \times 100 \%$$

Where:
Nsns = number of strong normal seedlings
Nst = number of seeds tested

Meanwhile, the incompatibility level based on the number of normal seedlings, was determined by calculating the number of normal seedlings seven days after planting, using the following formulation:

$$\% \text{ of normal seedlings} = \frac{\text{Nns}}{\sum \text{Nst}} \times 100 \%$$

Where:
Nns = number of normal seedlings
∑ Nst = total number of seeds tested

RESULTS AND DISCUSSION

The Comparison between Incompatibility Level Based on Percentages of Fruit Sets and Normal Seedlings

Evaluation of incompatibility levels of the crossing based on percentages of fruit sets and normal seedlings (Table 1). The result showed that 3,120 cross-pollination produced 659 fruit sets, 767 seeds with 509 seeds contain an embryo, 250 growths to be normal seedlings. There were total of 5,188 crossing on 32 cross-combination with reciprocal produced 1,287 fruit sets, 1,700 seeds with 1,148 seeds contain embryo, 506 growth to be normal seedlings (Table 1 and Table 2). Almost all cross-combination produced both fruit and seeds, except for the combination of C x K and H x J. This was caused by pollen germination failure that leads to fertilization failure. Cross-pollination in hexaploid types of plants was seen to be less likely to produce fruit set and seed, because of the incompatibility and sterility issues which were caused by unbalance in segregation and gene recombination during the meiotic process (Martin, 1981).

Cross combination was compatible when evaluated based on the fruit set, but based on the percentage of normal seedlings was categorized as partial compatible, very incompatible, or perhaps fully incompatible. In this case, the formed fruits may not produce an embryo, and the embryo may not result in normal seedlings. For instances, crossing between clones of D x K and F x I was compatible based on fruit set but fully incompatible based on percentages of normal seedling because it could not produce normal seedlings (Table 1).

Based on the percentage of normal seedlings, the observed sweetpotato clones had different characters based on its ability as female or male parents. Clone B (Papua Solossa) turned out to be more compatible as a female parent because it was compatible with the other four male parents. Meanwhile, the clone of I (Beta-2) is the most suitable as male parent, as this clone was compatible to six of eight female parent clones (Table 1). This resonates with the experiment by Susanto, Sulasmi, & Rahayuningsih (2013) which found that Papua Solossa clone is classified as sporophytic self- incompatible. The pistil receptivity of this clone shows 100 % which means the clone is more suitable as a female parent.

The Comparison of Incompatibility Level Based on Percentages of Fruit Sets and Normal Seedlings on Reciprocal Crossing

Evaluation of incompatibility levels of the reciprocal crossing based on the percentages of fruit sets and normal seedlings is shown in Table 2. In reciprocal crossing, about 2,068 cross-pollinations produced 30.4 % or as many as 628 fruit sets, 955 seeds and 639 seeds containing embryos (average seed/fruit ranged 0-2.3), furthermore 256 grown to be normal seedlings. Seed from controlled sweetpotato crossings has especially high value (1-3 seeds per fruit) are obtained from a successful pollination (Grüneberg et al., 2015).

Almost all crossings produced seed, but not all seed contained embryo. For example, a combination of J x B, which produced 25 seeds, mainly without an embryo. This shows that the incompatibility occurs during the fertilization process. It means that neither an embryo nor endosperm is formed. Also, pollen sterility and incompatibility within the pollen-stigma interaction also contribute to the low success rate of fruit set production in sweetpotato (Lestari, 2010). Failures of reproductive processes are due to the male and female gametes abnormality, failure germination on some pollen and failure on stimulation germination by stigma. Furthermore, pollen tubes do not pass from stigma to style and entire style, so pollen tubes do not result in fertilization, ovules develop as scales, seeds do not develop normal endosperms and finally seeds do not germinate, seedlings are weak and die, seedlings do not grow into mature plants (Martin, 1981).

Incompatibilities which occur before pollination, being pollination and post-pollination indicate a very complex mechanism both saprophytically and gametophytically causing failure on normal seedling production. For example crossing between clones of J x D produced 25 % fruit sets (compatible based on % fruit set), the fruit sets contained 16 seeds but without embryo, so percentages of normal seedlings were 0 (fully incompatible based on % normal seedling). According to this data, the incompatibility level estimation based on the normal seedling percentage is likely to be a more valid indicator. Beside, this method could identify the offspring which produce new clones.

According to the normal seedlings growth on reciprocal cross-pollination, clone K was observed as the most suitable female parent (on reciprocal crosses), because this clone was compatible with the six male parents as seen in Table 2. Clone K as male parent, was only compatible with three female parents (Table 1). This indicates that stigma of clone K contains exudates that compatible with pollen growth and development. Thus, this clone is more suitable as a female parent.

Table 1. The comparison level of incompatibility based on percentage of fruit sets and normal seedlings

Crossing		Number of crossing	Number of Fruit set	% of Fruit set	Number of Seed	Average seed/fruit	Number of seed filled	Number of normal seedlings	% of Normal seedlings	Level of incompatibility based on	
Female	Male									% fruit sets	% normal seedlings
A	I	43	9	20.9	12	1.3	11	8	72.7	C	C
	J	96	11	11.5	12	1.1	0	0	0.0	PI	FI
	K	76	17	22.4	17	1.0	8	1	12.5	C	PC
	L	35	12	34.3	17	1.4	10	1	10.0	C	PC
B	I	51	19	37.3	33	1.7	21	18	85.7	C	C
	J	63	45	71.4	93	1.1	48	28	58.3	C	C
	K	93	33	35.5	50	1.5	27	14	51.9	C	C
	L	91	17	18.7	21	1.2	9	5	55.6	PC	C
C	I	68	20	294	23	1.1	13	5	38.5	C	C
	J	91	41	45.1	54	1.3	20	15	75.0	C	C
	K	68	0	0.0	0	0	0	0	0.0	FI	FI
	L	79	3	3.8	3	1.0	0	0	0.0	VI	FI
D	I	117	23	19.7	32	1.3	25	17	68.0	PC	C
	J	57	33	57.9	44	1.3	28	19	67.9	C	C
	K	58	13	22.4	13	1.0	0	0	0.0	C	FI
	L	92	30	32.6	40	1.3	35	22	62.9	C	C
E	I	123	17	13.8	11	0,6	7	4	57.1	PC	C
	J	120	8	6.7	8	1.0	0	0	0.0	VI	FI
	K	126	5	4.0	5	1.0	0	0	0.0	VI	FI
	L	174	22	12.6	23	1.0	17	4	23.5	PC	C
F	I	48	13	27.1	19	1.4	13	0	0.0	C	FI
	J	54	16	29.6	22	1.3	13	2	15.4	C	PC
	K	36	3	8.3	3	1.0	0	0	0.0	I	FI
	L	52	4	7.7	6	1.5	0	0	0.0	I	FI
G	I	118	22	18.6	0	0	0	0	0.0	PC	FI
	J	146	28	19.2	40	1.4	28	7	25.0	PC	C
	K	140	22	15.7	27	1.2	25	5	20.0	PC	C
	L	97	12	12.4	14	1.1	12	1	8.3	PC	VI
H	I	145	53	36.6	15	0.3	55	32	58.2	C	C
	J	169	0	0.0	0	0	0	0	0.0	FI	FI
	K	175	52	29.7	69	1.3	43	24	55.8	C	C
	L	219	56	25.6	41	0.7	41	18	43.9	C	C
		3120	659		767		509	250			

Remarks: Female: Kidal (A), Papua Solossa (B), Ir. Melati (C), Cilembu-1 (D), UJ-16 Slape (E), MSU 07022-12 (F), MSU 07023-86 (G), MSU 07015-54 (H); Male: Beta-2 (I), MSU 07022-14 (J), MSU 07009-12 (K) and RIS 10068-02 (L); C=compatible; PC=Partial compatible; VI=Very incompatible; FI=Fully incompatible

Table 2. The comparison level of incompatibility level based on a percentage of fruit sets normal seedlings on a reciprocal crossing

Crossing		**Number of crossing**	**Number of fruit set**	**% of fruit set**	**Number of seed**	**Average seed per fruit**	**Number of seed filled**	**Number of normal seedling**	**% of normal seedlings**	**Level of incompatibility based on**	
Female	**Male**									**% fruit set**	**% normal seedlings**
I	A	65	22	33.8	26	1.2	16	2	12.5	C	PC
	B	31	13	41.9	21	1.6	8	2	25.0	C	C
	C	44	0	0.0	0	0.0	0	0	0.0	FI	FI
	D	31	6	19.4	10	1.7	0	0	0.0	PC	FI
	E	109	2	1.8	2	1.0	16	0	0.0	VI	FI
	F	76	27	35.5	42	1.6	27	14	51.9	C	C
	G	75	3	4.0	0	0.0	0	0	0.0	VI	FI
	H	37	17	45.9	39	2.3	17	13	76.5	C	C
J	A	75	26	34.7	34	1.3	25	7	28.0	C	C
	B	94	16	17.0	25	1.6	0	0	0.0	PC	FI
	C	70	18	25.7	12	0.7	8	6	75.0	C	C
	D	56	14	25.0	16	1.1	0	0	0.0	C	FI
	E	81	29	35.8	47	1.6	32	14	43.8	C	C
	F	89	31	34.8	44	1.4	37	15	40.5	C	C
	G	67	21	31.3	29	1.4	19	3	15.8	C	PC
	H	57	34	59.6	56	1.6	39	13	33.3	C	C
K	A	93	39	41.9	51	1.3	30	9	30.0	C	C
	B	54	24	44.4	33	1.4	15	8	53.3	C	C
	C	49	1	2.0	2	2.0	0	0	0.0	VI	FI
	D	64	27	42.2	45	1.7	21	7	33.3	C	C
	E	50	24	48.0	26	1.1	19	8	42.1	C	C
	F	77	3	3.9	5	1.7	0	0	0.0	VI	FI
	G	77	34	44.2	38	1.1	38	9	23.7	C	C
	H	80	33	41.3	44	1.3	44	15	34.1	C	C
L	A	57	20	35.1	32	1.6	28	5	17.9	C	PC
	B	48	31	64.6	47	1.5	19	7	36.8	C	C
	C	39	1	2.6	1	1.0	0	0	0.0	VI	FI
	D	67	23	34.3	44	1.9	38	27	71.1	C	C
	E	96	57	59.4	109	1.9	94	51	54.3	C	C
	F	73	2	2.7	0	0.0	0	0	0.0	VI	FI
	G	61	19	31.1	35	1.8	31	15	48.4	C	C
	H	26	11	42.3	18	1.6	18	6	33.3	C	C
Total		2068	628		933		639	256			

Remarks: Female: Kidal (A), Papua Solossa (B), Ir. Melati (C), Cilembu-1 (D), UJ-16 Slape (E), MSU 07022-12 (F), MSU 07023-86 (G), MSU 07015-54 (H); Male: Beta-2 (I), MSU 07022-14 (J), MSU 07009-12 (K) and RIS 10068-02 (L); C=compatible; PC=Partial compatible; VI=Very incompatible; FI=Fully incompatible

Table 3. Correlation between of incompatibility level and percentages of seed vigour

Crossing		Level of	%	Reciprocal crossing		Level of	%
Female	**Male**	**Compatibility**	**Vigour**	**Female**	**Male**	**Compatibility**	**Vigour**
A	I	Compatible	54.5	I	A	Partial compatible	6.3
	J	Fully incompatible	0	J		Compatible	20
	K	Partial compatible	12.5	K		Compatible	23.3
	L	Partial compatible	0	L		Partial compatible	17.9
B	I	Compatible	71.4	I	B	Compatible	25
	J	Compatible	50	J		Fully incompatible	0
	K	Compatible	44.4	K		Compatible	46.7
	L	Compatible	44.4	L		Compatible	36.8
C	I	Compatible	38.5	I	C	Fully incompatible	0
	J	Compatible	60	J		Compatible	75
	K	Fully incompatible	0	K		Fully incompatible	0
	L	Fully incompatible	0	L		Fully incompatible	0
D	I	Compatible	68	I	D	Fully incompatible	0
	J	Compatible	67.9	J		Fully incompatible	0
	K	Fully incompatible	0	K		Compatible	28.6
	L	Compatible	57.1	L		Compatible	68.8
E	I	Compatible	57.1	I	E	Fully incompatible	0
	J	Fully incompatible	0	J		Compatible	40.6
	K	Fully incompatible	0	K		Compatible	42.1
	L	Partial incompatible	17.6	L		Compatible	51.1
F	I	Fully incompatible	0	I	F	Compatible	51.9
	J	Partial compatible	15.4	J		Compatible	40.5
	K	Fully incompatible	0	K		Fully incompatible	0
	L	Fully incompatible	0	L		Fully incompatible	0
G	I	Fully incompatible	0	I	G	Fully incompatible	0
	J	Partial incompatible	17.9	J		Partial compatible	15.8
	K	Partial incompatible	16	K		Partial compatible	15.8
	L	Very incompatible	8.3	L		Compatible	48.4
H	I	Compatible	25.5	I	H	Compatible	76.5
	J	Fully incompatible	0	J		Compatible	33.3
	K	Compatible	46.5	K		Compatible	29.5
	L	Compatible	39	L		Compatible	33.3

Remarks: Female: Kidal (A), Papua Solossa (B), Ir. Melati (C), Cilembu-1 (D), UJ-16 Slape (E), MSU 07022-12 (F), MSU 07023-86 (G), MSU 07015-54 (H); Male: Beta-2 (I), MSU 07022-14 (J), MSU 07009-12 (K) and RIS 10068-02 (L)

The Correlation between incompatibility level and seed vigour

The incompatibility level of sweetpotato from a controlled cross-pollination determines the seedling growth vigour. In a compatible cross-pollination, a normal seedling generally has growth vigour of more than 20 %. This compatible seedling also had a better growth vigour than those of incompatible cross-pollination combinations. This research revealed eight cross-pollination combinations and their reciprocal crosses which were compatible and had quite high growth vigour, for example cross combination of L x D (Figure 4). Those clones are highly recommended as cross-pollination parents because, they were able to produce seeds and normal seedlings. However, the superior characters of parents are also important to be included other than normal seedlings production only. Five cross-pollinations and their reciprocal crosses were fully incompatible. This shows their unsuitability for cross-pollination combination since they were not able to produce normal seedlings, as seen in Table 3.

Genetically, the compatibility of cross-combination shows compatibility between the pollen-stigma interaction. This compatibility is indicated by germination of pollen on the stigma surface, the development of pollen tube, and finally the fertilization process in the *ovary*. On the other hand, the incompatible cross-pollination combination indicates that there were some failures in some seed development stages beside external factors during the seed development that strongly influences the seeds growth vigour. Physiologically, Ichsan (2006) argued that the seed growth vigour was determined by a metabolic process during the seed development stages, from the pollination process to the seed maturity process.

CONCLUSION

The use of normal seedling percentage as a new parameter in evaluating cross-pollination has apparently seemed to be more effective than the fruit set percentage method because the numbers of new clones could be known accurately. There were 5,188 times of cross-pollination and their reciprocal produced 506 normal seedlings (about 10 %).

Papua Solossa more compatible as a female parent and Beta-2 was the most suitable as male parent. This information is important to get more compatibility of crossing for the future.

Seed vigour can be used as a physiological marker for comparing the level of incompatibility on sweetpotato crossing.

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REFERENCES

Basuki, N. (1986). *Pendugaan parameter genetik dan hubungan antara hasil dengan beberapa sifat agronomis serta analisis persilangan diallel pada ubi jalar (Ipomoea batatas* (L.) Lamb.*)* [The estimation of genetic characters and relationship between yield and agronomical characters and analysis of diallel mating on sweet potato (*Ipomoea batatas* (L.) Lamb)] (Doctoral dissertation). Retrieved from http://repository.ipb.ac.id/bitstream/handle/123456789/1338/1986nba.pdf?sequence=4&isAllowed=y

Filho, J. M. (2015). Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola, 72*(4), 363-374. http://doi.org/10.1590/0103-9016-2015-0007

Gasura, E., Mashingaidze, A. B., & Mukasa, S. B. (2008). Genetic variability for tuber yield, quality, and virus disease complex traits in uganda sweetpotato germplasm. *African Crop Science Journal, 16*(2), 147-160. Retrieved from http://www.bioline.org.br/pdf?cs08017

Grüneberg, W. J., Ma, D., Mwanga, R. O. M., Carey, E. E., Huamani, K., Diaz, F., ... Yencho, G. C. (2015). Advances in sweetpotato breeding from 1992 to 2012. In J. Low, M. Nyongesa, S. Quinn, M. Parker (Eds.), *Potato and sweetpotato in Africa: transforming the value chains for food and nutrition security* (pp. 3-68). Wallingford, UK: CAB International.

Gurmu, F., Hussein, S., & Laing, M. (2013). Self- and cross-incompatibilities in sweetpotato and their implications on breeding. *Australian Journal of Crop Science, 7*(13), 2074-2078. Retrieved from http://www.cropj.com/gurmu_7_13_2013_2074_2078.pdf

Ichsan, C. N. (2006). Uji viabilitas dan vigor benih beberapa varietas padi (*Oryza sativa* L.) yang diproduksi pada temperatur yang berbeda selama kemasakan [Viability test and Seed vigour some paddy varieties (*Oryza sativa* L.) which produced on different temperature for maturating]. *Jurnal Floratek, 2*(1), 37-42. Retrieved from http://jurnal.unsyiah.ac.id/floratek/article/view/70

Jayasuriya, K. M. G. G., Baskin, J. M., Geneve, R. L., & Baskin, C. C. (2007). Morphology and anatomy of physical dormancy in *Ipomoea lacunosa*: Identification of the water gap in seeds of convolvulaceae (Solanales). *Annals of Botany*, *100*(1), 13–22. http://doi.org/10.1093/aob/mcm070

Jones, A. (1980). Sweet potato. In W. R. Fehr, & H. H. Hadley (Eds), *Hybridization of crop plants* (pp. 645-655). Madison, USA: American Society of Agronomy-Crop Science Society of America.

http://doi.org/10.2135/1980.hybridizationofcrops.c46

Kowyama, Y., Shimano, N., & Kawase, T. (1980). Genetic analysis of incompatibility in the diploid Ipomoea species closely related to the sweet potato. *Theoretical and Applied Genetics, 58*(3-4), 149-155. http://doi.org/10.1007/BF00263108

Kowyama, Y., Tsuchiya, T., & Kakeda, K. (2000). Sporophytic self-incompatibility in *Ipomoea trifida*, a close relative of sweet potato. *Annals of Botany*, *85*, 191–196. http://doi.org/10.1006/anbo.1999.1036

Lestari, S. U. (2010). Pengaruh inkompatibilitas dan sterilitas terhadap pembentukan kapsul dan biji ubijalar (Effect of incompatibility and sterility to fruit and seed set of sweetpotato). *AGRIVITA Jurnal tentang Ilmu-Ilmu Pertanian, 32*(1), 19-28.

Martin, F. W. (1965). Incompatibility in the sweet potato: A review. *Economic Botany, 19*(4), 406-415. Retrieved from https://www.jstor.org/stable/4252651?seq=1#page_scan_tab_contents

Martin, F. W. (1981). Analysis of the incompatibility and sterility of sweet potato. In R. L. Villareal, & T. D. Griggs (Eds.), *Sweet potato (AVRDC)* (pp. 275-283). Proceedings of the First International Symposium, Taiwan.

Martin, F. W., & Cabanillas, E. (1966). Post-pollen-germination barriers to seed set in sweet-potato. *Euphytica, 15*(3), 404-411. http://doi.org/10.1007/BF00022187

Martin, F. W., & Jones, A. (1986). Breeding sweet potatoes. In F. A. Bliss, R. J. Dinus, & J. W. Dudley (Eds.), *Plant breeding reviews* (vol. 4), (pp. 313-346). Wesport, USA: AVI Publishing Company.

Moriya, L. M., Neto, N. B. M., Marks, T. R., & Custódio, C. C. (2015). Seed vigour better to be assessed by physiological markers rather than expression of antioxidant enzymes in the common bean (*Phaseolus vulgaris* L.). *Australian Journal of Crop Science*, *9*(1), 30–40. Retrieved from https://www.researchgate.net/publication/273769471_Seed_vigour_better_to_be_assessed_by_physiological_markers_rather_than_expression_of_oxidant_enzymes_in_the_common_bean_Phaseolus_vulgaris_L

Sadjad, S. (1994). *Kuantifikasi metabolisme benih* [Kuantification of seed metabolism]. Jakarta, ID: Grasindo.

Susanto, F. A., Sulasmi, E. S., & Rahayuningsih, St. A. (2013). Pollen morphology and sucrose giving on stigma relation with incompability of sweetpotato (*Ipomoea batatas* (L.) L.). *The Journal of Tropical Life Science, 3*(3), 207-211. Retrieved from http://www.jtrolis.ub.ac.id/index.php/jtrolis/article/view/92

Wang, H. (1964). A study on the self- and cross-incompatibilities in the sweet potato in Taiwan (Formosa). *Proceeding of the American Society for Horticultural Science, 84*, 424-430.

Wang, H. (1968). A study of fruit and seed setting ability and female sterility in the sweetpotato (*Ipomoea batatas* (L.) Lam). *Botanical Buletin of Academica Sinica, 9*, 139-150.

Wang, H. (1975). The breeding and cultivation of sweet potatoes. *Technical Bulletin (Asian and Pacific Council, Food, & Fertilizer Technology Center)* no. 26, 1-41.

Wheeler, M. J., Franklin-Tong, V. E., & Franklin, F. C. H. (2001). The molecular and genetic basis of pollen-pistil interactions. *New Phytologist*, *151*(3), 565–584. http://doi.org/10.1046/j.0028-646x.2001.00229.x

Zuraida, N., & Supriati, Y. (2001). Usahatani ubi jalar sebagai bahan pangan alternatif dan diversifikasi sumber karbohidrat [Sweet potato farming as sources for food alternative and carbohydrate diversification]. *Buletin AgroBio, 4*(1), 13-23. Retrieved from http://biogen.litbang. Pertanian.go.id/terbitan/pdf/agrobio_4_1_13-23.pdf