Turkish Online Journal of Qualitative Inquiry (TOJQI)

Volume 12, Issue 5, June 2021: 763 - 788

Research Article

The crossbreeding compatibility of Kenaf (Hibiscus cannabinus L.) with its close relatives

Parnidi[1], Lita Soetopo[1], Damanhuri[1] and Marjani[2]

Abstract

Hybridization is one of the breeding methods for kenaf (*Hibiscus cannabinus* L.) that can generate superior varieties. This research investigated interspecific hybrids between kenaf and its close relatives. The research was conducted in April–October 2018 at the experimental garden of Balittas Karangploso, Malang, Indonesia. The research materials were eight genotypes of *H. cannabinus* (KR1, KR4, KR5, KR6, KR15, Kin 2, DS028, and Cuba 108/Italia), and three of its close relatives (*H. radiatus* (Kal II), *H. acetocella* (SSRH 023), and *H. sabdariffa* (HS40)). The proportion of successful kenaf interspecific hybrids was 0–97.92%. The interspecific hybrids between *H.cannabinus* as the parental female and *H. radiatus* as the parental male were compatible (100%). Interspecific hybrids between *H. cannabinus* as the parental female and *H. acetocella* as the parental male were very compatible (12.5%), partly compatible (75%), and incompatible (12.5%). Interspecific hybrids between *H. cannabinus* as the parental female and *H. sabdariffa* as the parental male were compatible (12.5%), partly compatible (50%), and incompatible (37.5%). A reciprocal cross between *H. cannabinus* and *H. acetocella* was compatible (100%), but a reciprocal cross between *H. cannabinus* and *H. sabdariffa* was compatible (75%) and incompatible (25%). The mean viability of seed from the interspecific hybrids between *H. cannabinus* and its three close relatives was 0%. The viability of seed obtained from the reciprocal cross between kenaf and *H. radiatus* was 41.5%, between kenaf and *H. acetocella* was 31.5%, and between kenaf and *H. sabdariffa* was 0%.

Keywords: *H. cannabinus*, close relatives, Interspecific hybrids.

(1) Agriculture Faculty - Brawijaya University of Malang
(2) Sweetener and Fiber Crops Research Institute
e-mail: 1. nikicro@yahoo.co.id

INTRODUCTION

H. cannabinus (also known as kenaf) is a plant in the Malvaceae family and is a well-known fiber producer that is widely used. The kenaf fibers are obtained from the stalk bark and have a high economic value. The *H. cannabinus* varieties that have been developed in Indonesia have high fiber production, but they are not resistant to biotic and abiotic problems. Interestingly, other kenaf relatives from *Hibiscus* sp. are widely known to have a resistance toward biotic and abiotic problems. For example, *H. radiatus*, *H. acetocella*, and *H. sabdariffa* are resistant to root-knot nematodes. *H. Acetocella*, which is widely distributed in the potsolid red-yellow South Kalimantan (Borneo), tolerates low soil pH (Hartati. 2004).

Interspecific hybrids of *Hibiscus* sp. have been cultivated by plant breeders to generate superior varieties that have a high fiber content but are also resistant to biotic and abiotic problems (Basavaraja et al. 2018; Szymadja et al. 2015, Ocampo et al. 2016, Giovannini et al. 2012). Breeding interspecific hybrids is one way to enhance the genetic diversity of Kenaf. Knowledge of interspecific hybrids of kenaf is useful in selecting a method to produce F1 hybrids and to trace relative relationships among species. One problem with using interspecific hybrids is their incompatibility (Hartati. 2004, Satya et al. 2013), which is caused by a prezygotic barrier (whether the pollen germinates in the stigma) and a postzygotic barrier (whether the fruit sets develop after the pollination) (Ocampo et al. 2016). The incompatibility mechanism makes it difficult for the plant breeder to produce hybrid seeds. However, several previous studies have shown that there was a compatibility between *H. cannabinus* and its close relatives.

Satya et al. (2012) reported the success of interspecific hybrids between *H. cannabinus* and *H. radiatus* with a cross efficiency of 6.41%. However, the reciprocal cross between *H. cannabinus* and *H. radiatus* was only 1.67%. Hartati (2004) succeeded in producing interspecific hybrids between *H. cannabinus* and *H. radiatus*. Colchicine was given to the F1 plants so that the F1 plants could produce fertile seeds. Ghosh and Sanyal (1960) reported that offspring (F4) of the interspecific hybrid between *H. cannabinus* and *H. radiatus* had good resistance to plant disease. In addition, Manzel and Wilson (1967) reported that the hybrid between *H. acetocella* and *H. radiatus* generated hybrid offspring (F2), which were vigorous and had seed. Arangzeb (1996) found that the hybrid between *H. acetocella* × F1 (*H. radiatus* × *H. cannabinus*) generated hybrid offspring (F3), which were resistant to nematodes and were unbranched.

A further investigation of the compatibility of interspecific hybrids of kenaf is crucial to maximizing the seed yield for multiplication materials. The compatibility of kenaf interspecific hybrids has not been thoroughly explored. To address this lack of knowledge, this research aimed to investigate the degree of compatibility of interspecific hybrids of *H. cannabinus* and its close relatives *H. radiatus*, *H. acetocella*, and *H. sabdariffa*.

RESEARCH MATERIALS AND METHODS

This research was conducted in April to October 2018 at the experimental garden of Balittas Karangploso, Malang, Indonesia. All plants were treated equally. The research materials were eight genotypes of *H. cannabinus* (KR1, KR4, KR5, KR6, KR15, Kin 2, Cuba 108/Italia, and DS028) and one genotype each of its three close relatives *H. radiatus* (Kal II), *H. acetocella* (SRRH 023), *H. sabdariffa* (HS40). The flower characteristics of these plants are shown in Table 1. Other materials used were urea fertilizer, Phonska, herbicide, insecticide, fungicide, paper bags, tweezers, cutters, thread, paper clips, labels, plastic straws, and plastic.

Each genotype was planted in a 5 m × 5 m seedbed with a planting distance of 80 × 25 cm, and the distance between each seedbed was 1 m. The fertilizer used in this research was 180 kg of N and 45 kg of P_2O_5 per ha, which was equivalent to 300 kg urea and 300 kg of Phonska per ha. The Phonska fertilizer was applied to the plant ten days after planting (DAP). The urea fertilizer was applied at 30 DAP. Other plant maintenance involved weeding, watering, and controlling pests according to the condition of the plants. The herbicide, insecticide, and fungicide were also applied during this step. The interspecific hybrids consisted of 24 combinations, 20 reciprocal crosses, and 11 self-pollinations.

The hybridization method followed Marjani (2015). Emasculation was performed one day before the flower bloomed to prevent self-pollination. The emasculation was carried out on normal flower buds during the afternoon and early evening (between 1 p.m. and 6 p.m.). Emasculation was performed by slicing the flower buds on part of the corolla and removing the stem so that two-thirds of the stamen was exposed. Then, using tweezers, the stamen was discarded carefully. After clearing the stamen, the flower buds were covered by paper bags to avoid pollen contamination carried by the wind or insects. A paper bag also covered the anther in plants used as the male parent.

The artificial pollination was conducted in the morning (between 6 a.m. and 9 a.m.) by attaching the anther to the emasculated stigma, covering it with a paper bag, and labeling it. The seed was harvested if the fruit had already ripened, and the fruit peel had dried and become a brownish color. The seeds were collected from the fruit and sundried until the water content was 7–8%. Successful hybrids were made on 30 inbred flowers and each fruit to know the percentage of full and empty seed.

Observation Variables:

Pollen Fertility

The pollen fertility was measured by staining with iodium-potassium-iodide. The pollen fertility was measured using preparate under a microscope and counting the fertile pollen. Under microscopic observation, the fertile pollen was dark or black, but the sterile pollen was transparent (Brewbaker. 1957). The pollen fertility was categorized according to the percentage of fertile pollen in the sample: fertile (61–100%), partly fertile (31–60%), partly sterile (11–30 %), and sterile (0–10%).

Determination of the success of pollination was carried out by counting flowers that were pollinated successfully in the 1–3 days after pollination. Swanson et al. (2008) stated that the flowers that failed to pollinate would fall off in the following 22–24 hours. The seed was harvested after the capsule was physiologically ripe (yellow and dry).

Fruit Development

After hybridization, the hybridization success was determined as the percentage of fertilized flowers in the total number of hybrid flowers. The fruit was harvested when it was physiologically ripe (brownish fruit peel), and the seeds of the fruit were studied.

The Number of Seeds per Fruit

The seeds from the fruits were counted manually.

The Compatibility of Interspecific Hybrids

Wang's classification (1964) was used to determine whether an interspecific hybrid was compatible or incompatible. The compatibilities were classified by the formation of capsules: compatible (> 20%), partly incompatible (10–20%), and fully incompatible (no capsule formed). The data was analyzed using Microsoft Excel to determine the percentage of successful hybrids.

The Viability of Seed

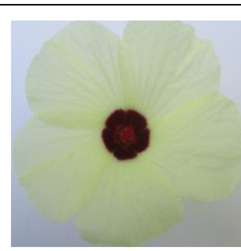
Fifty seeds were tested on a straw paper substrate to determine the viability of the seed. Germination was observed for seven days. The germination rate was calculated as a percentage from the number of germinated seeds and the total number of seeds in each sample.

Table 1. Flower characteristics of *H. cannabinus* L. and its close relatives.

No	Genotypes	Images	Characteristics of flower color
1.	*H. radiatus (Kal II)*		Corolla pink Anther brown
2.	*H. acetocella (SSRH 023)*		Corolla purple Anther yellow
3.	*H. sabdariffa (HS40)*		Corolla Creamy Anther brown
4.	*H. cannabinus* L. *(KR 1)*		Corolla white Anther yellow

5.	*H. cannabinus* L. *(KR 4)*		Corolla creamy Anther brown
6.	*H. cannabinus* L. *(KR 5)*		Corolla creamy Anther brown

Continued

7.	*H. cannabinus* L. *(KR 6)*		Corolla creamy Anther brown
8.	*H. cannabinus* L. *(KR 15)*		Corolla creamy Anther brown

9.	*H. cannabinus* L. *(Kin 2)*		Corolla creamy Anther brown
10.	*H. cannabinus* L. *(DS028)*		corolla creamy Anther brown
11.	*H. cannabinus* L. *(Cuba/Italia 108)*		Corolla creamy Anther brown

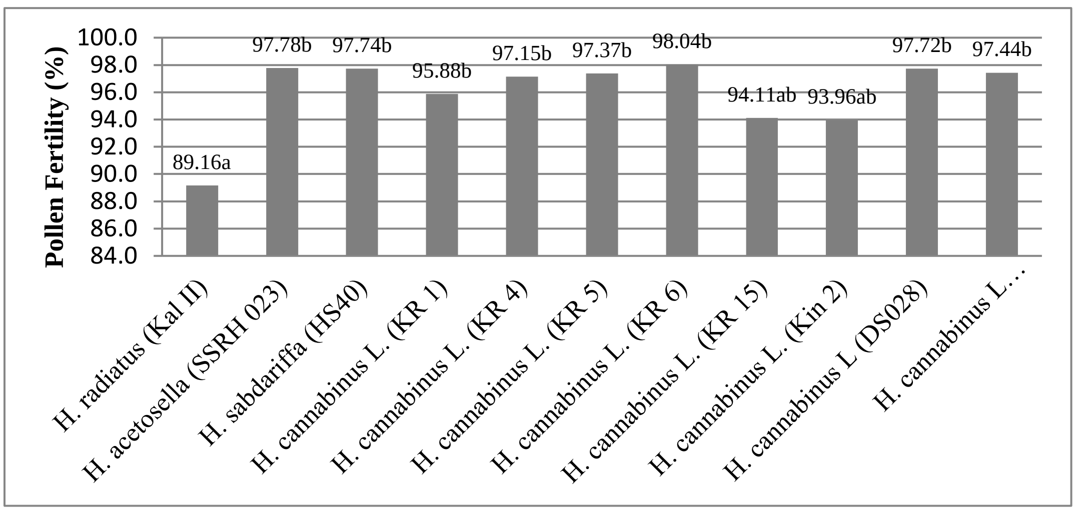
RESULTS

Pollen Fertility

The pollen produced by eight genotypes of *H. cannabinus* with its three close relatives had a variety of sizes (small, medium, and large), shapes (fully round and partly round), and colors (dark and transparent). Pollen fertility tests found that the mean pollen fertility was 89.2–98.0%. Fertile pollen was indicated by a dark color, a normal size, and an identical shape, whereas sterile pollen was small, abnormal in shape, and light in color. The highest pollen fertility (98.0%) was found for genotype KR6.

Genotype KR 5 had the largest pollen (mean diameter: 61.81 μm), and genotype KR 15 had the smallest pollen (mean diameter: 52.03 μm). The pollen of genotypes Kal II, KR 1, and KR 15 had more varied sizes, shapes, and colors than the other genotypes. The color of the fertile pollen of Kal II was lighter than the fertile pollen of the other genotypes.

Figure 1. Pollen fertility of *H. cannabinus* L. and its close relatives as parents.



Note: Different letters indicating a significant difference (P<0.05).

Fruit Development

The observation of interspecific hybrids began on the third day after pollination and continued until harvest time. Each combination of *H. cannabinus* hybrid had a different capability for producing fruits and seeds (Table 3 in the Appendix). The fertilized flowers appeared to be fresher and more strongly attached to the stalk than the unfertilized flowers. The unfertilized flowers were withered and fell off. The fruit set grew visibly from the third day after pollination. The fruit development of both interspecific hybrids between kenaf and its close relatives and the reciprocal crosses showed imperfect development. The fruits produced were smaller and wrinkled. Whereas, the fruits produced by self-pollination appeared better. The fruit was harvested when it had ripened physiologically; this was 6–7 weeks after pollination.

The eight genotypes of *H. cannabinus* and its three close relatives were self-compatible (see Table 1). The mean percentage of fruit sets and seed produced from interspecific hybrids between *H. cannabinus* and its relatives was lower than for the self-pollinated plants. The mean percentage of fruit sets and seed produced from self-pollination was 75%.

The mean number of fruit sets produced by eight interspecific hybrid combinations of *H. cannabinus* (parental female) × *H. radiatus* (parental male) was 38.75 fruits. The mean percentage of successful pollination was 68.56%, and the mean number of seeds per fruit was 11.89 seeds. The mean number of fruit sets produced from eight interspecific hybrid combinations of *H. cannabinus* (parental female) × *H. acetocella* (parental male) was 11.62 fruits. The mean percentage of successful pollination was 17.73%, and the mean number of seeds per fruit was 6.77 seeds. The mean number of fruit sets produced by eight interspecific hybrid combinations of *H. cannabinus* (parental female) × *H. sabdariffa* (parental male) was 6.87 fruits. The mean percentage of successful pollination and the mean number of seeds per fruit were 13.09% and 3.41 seeds, respectively.

The mean number of fruit sets produced from reciprocal crosses of *H. cannabinus* (parental male) × *H. radiatus* (parental female) was 41.50 fruits. The mean percentage of successful pollination and the mean number of seeds per fruit were 97.35% and 15.25 seeds, respectively. The mean number of fruit sets produced from a reciprocal cross of *H. cannabinus* (parental male) × *H. acetocella* (parental female) was 46.50 fruits. The mean percentage of successful pollination and the mean number of seeds per fruit were 62.58% and 13.58 seeds, respectively. The mean number of fruit sets produced from a reciprocal cross of *H. cannabinus* (parental male) × *H. sabdariffa* (parental female) was 9.40 fruits. The mean percentage of successful pollination and the mean number of seeds per fruit were 25.33% and 0 seeds (seedless), respectively.

Classification of Compatibility Type

The percentage of fruit sets was used to classify hybrid parentals based on their compatibility or incompatibility, following Wang (1964). Of the 54 sets of hybrid combinations (see Table 2), 25 were compatible (>20%), seven partly incompatible (10–20%), four very incompatible (<10%), and 18 fully incompatible (fruitless).

The compatibilities of interspecific hybrids between *H. cannabinus* (parental female) and *H. radiatus*, *H. acetocellaI*, and *H. sabdariffa* (parental male) were 68.57%, 17.74%, and 13.09%, respectively. The compatibilities of the reciprocal cross between *H. cannabinus* (parental male) and *H. radiatus*, *H. acetocella*, and *H. sabdariffa* (parental female) were 97.36%, 62.58%, and 25.33%, respectively.

Table 2. The compatibility of interspecific hybrids between *H. cannabinus* L. and its close relatives based on fruit set.

♂ / ♀	Kal II	SSRH023	HS40	KR1	KR4	KR5	KR6	KR15	Kin2	DS028	Cuba italia 108
Kal II	C	C	FI	C	C	C	C	C	C	C	C
SSRH 023	C	C	FI	C	C	C	C	C	C	C	C
HS40	PC	FI	C	C	C	C	C	VI	C	C	VI
KR 1	C	PC	PC	C							
KR 4	C	PC	PC		C						
KR 5	C	PC	PC			C					
KR 6	C	VI	VI				C				
KR 15	C	PC	VI					C			
Kin 2	C	PC	VI						C		
DS028	C	C	C							C	
Cuba/Italia 108	C	PC	PC								C

Note: *Compatible* (C)=fruit set >20 %, *Partly Compatible* (PC)=fruit set 10–20%, Very Incompatible (VI)=fruit set <10%, and Fully Incompatible (FI)=fruitless (0%).

The Viability of Seeds

The germination rate was used to assess the seed viability (see Table 4 in the Appendix). Germination rate tests found that seeds started to germinate 2–7 days after planting. The mean germination rates of seeds from the interspecific hybrids between the eight genotypes of *H. cannabinus* and *H. radiatus*, *H. acetocella*, and *H. sabdariffa* was 0%. The mean germination rates of seeds produced by interspecific hybrids between *H. cannabinus* (parental female) and *H. radiatus*, *H. acetocella*, and *H. sabdariffa* (parental male) was 0%. In contrast, the germination rates of the reciprocal crosses were 24–60% (*H. cannabinus* × *H. radiatus*), 18–37.66% (*H. Cannabinus* × *H. acetocella*), and 0% (*H. cannabinus* × *H. sabdariffa)*.

DISCUSSION

All pollen produced from the interspecific hybrids between eight genotypes of *H. cannabinus* and its three close relatives was fertile. Pollen fertility is essentially an indication of the plant's ability to produce good pollen. Kurnia et al. (2017) suggested that fertile and compatible pollen is normal and round-size. Abnormal pollen is caused by imbalanced genetics. The imbalance separation of pollen caused by chromosome incompatibility leads to the size variation of pollen (Charles et al. 1974).

The results of this study found that the success of the interspecific hybrids between *H. cannabinus* and its close relatives was not just affected by pollen fertility. The pollen fertility was not positively correlated with the success of the interspecific hybrids between kenaf and its close relatives. Although the pollen fertility of each parental was high, the hybrids produced smaller fruits than the self-pollinated plants. Several combinations of interspecific hybrids between *H. cannabinus* and its close relatives were seedless, for example, *H. cannabinus* × genotype HS40 (see Table 1). These findings were consistent with those of Setiawati et al. (2016) and Kurnia et al. (2017), which confirmed that the success of interspecific hybrids of sweet potato was not affected by the level of pollen fertility.

The compatibility of pollination is likely to be affected by two processes: the pollen successfully fertilizes the ovule, and the fruit set is successfully formed after pollination (Charles et al. 1974, Plazas et al. 2016, Daunay et al. 2019). Vimala (1989) also suggested that pollen successfully fertilizes the ovule if the pollen is fertile. The rate of hybrid success also depends on the pollen fertilization on the stigma. The fertilized flowers appeared fresh, but the unfertilized flowers appeared dried three days after pollination

The incompatibility of interspecific hybrids is caused by prezygotic and postzygotic barriers (Satya et al. 2012, Ocampo et al. 2016). The prezygotic barrier leads to flower detachment before it develops into the fruit. This failure is caused by a failed germination and a late growth of the pollen tube. Fesenko et al. (2001) found that the pollen tube in an interspecific hybrid grew slower than in the intraspecific hybrid.

Szymadja et al. (2015) noted that if the pollen tube grew slowly, it led to low fruit production when it was crossed. It can also be assumed that the length of the pistils can affect the hybrid result. With long pistils, the pollen tubes need more time to fertilize than for short pistils. The low compatibility of interspecific hybrids between *H. cannabinus* and its close relatives was the result of a prezygotic barrier in the form of callose (Satya et al. 2012, Qin et al. 2012). Callose is the main component of the pollen tube wall, which can plug the pollen to fertilize the ovum. Satya et al. (2012) contended that callose in an interspecific hybrid was present because of the characteristics inherited from the parentals.

Mariola Plazas, Santiago Vilanova, Pietro Gramazio, Adri□

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and Francisco J. Herraiz

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and Francisco J. Herraiz

Mariola Plazas, Santiago Vilanova, Pietro Gramazio, Adri□

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and Francisco J. Herraiz

The postzygotic barrier in kenaf interspecific hybrids can occur at any stage, from the embryo to the adult plant. Several postzygotic barriers can inhibit zygote development and hybrid embryo formation. The postzygotic barrier has been found to result in a weak hybrid seed, which was undeveloped due to embryo miscarriage, the death of the F1 plant and the presence of chromosome elimination, a sterile hybrid plant, and low genetic recombination (Szymadja et al. 2015).

The rate of seed development in the kenaf interspecific hybrids was influenced by the species/genotype used for the hybridization. The results of this research found that the hybrid seeds produced had varied characteristics. The parental genotypes affect the hybrid embryo formation. Lestari and Deswiniyanti (2017) confirmed that the embryo formation of interspecific hybrids depended on the species/genotype of its parental because every species or genotype has different compatibility.

Species differences that affect the level of hybrid seed formation are also related to the degree of kinship of the parentals. The differences are shown by the group of the hybrid parental. The levels of seed formation produced from the interspecific hybrids between eight kenaf genotypes and *H. sabdariffa* were smaller than for the hybrids with *H. acetocella*, and *H. radiatus*. This result was consistent with the findings of Satya et al. (2015). They used ISSR and SSR markers to determine the level of kinship between *H. cannabinus, H. radiatus,* and *H. acetocella*, and the results of their crossing. Their results supported the proposal that the degree of kinship between *H. cannabinus* and *H. acetocella* was closer than between *H. cannabinus* and *H. radiatus.*

Species differences result in the different structures and morphology of chromosomes. These differences reduce the homology of chromosomes from the parentals. The number of chromosomes was 2n = 36 for *H. cannabinus* and 2n = 74 for *H. radiatus*, *H. acetocella*, and *H. sabdariffa*. The seeds produced from interspecific hybrids between kenaf and its close relatives were smaller than those produced from self-pollination. The different structure and morphology of chromosomes also affect the development of fruit and seeds. This was seen in the interspecific hybrids between kenaf and *H. sabdariffa* (parental male), which results in malformed fruit, but the interspecific hybrids between kenaf and *H. sabdariffa* (parental female) produced fruit with empty seeds.

The presence of a postzygotic barrier in interspecific hybrids between kenaf and its close relatives is also shown in the viability of the seed. The results of the viability tests demonstrated that with kenaf (parental female) and its close relatives (parental male) the seed viability was 0%. On the other hand, the viability of seeds from the reciprocal crosses was better than the plants with kenaf as parental female. This implied that the compatibility of interspecific hybrids between kenaf and its close relatives was greater for kenaf as the parental male than as the parental female.

The level of compatibility affects the viability of hybrid seeds; for example, the combination of parents (partially compatible × very incompatible) shows 0% germination. This is because the seeds do not contain embryos (they are empty), and the number and viability of the seeds produced are very low as they are unable to grow into normal sprouts.

Normal sprouts are generated by compatible combinations, which generally have higher viability than partly incompatible and very incompatible combinations. Similarly, Douglas and Freyrey (2016) concluded that *Nolana* plants that have high cross-compatibility resulted in high rates of seed germination. However, compatible combinations do not guarantee high viability. Some compatible combinations have low viability, and in some cases, the seeds are not even able to grow into normal sprouts. The low rate of seed germination produced from the interspecific hybrids between kenaf and its close relatives can be seen from the occurrence of wrinkle seeds. Wrinkle seeds occur because of damage to the embryo, empty embryos, or empty seeds.

If the compatibility level lies with the number of formed fruit, it is not possible to predict the number of normal sprouts that can be produced. The occurrence of formed fruits does not guarantee that seeds are present inside, that embryos form, or that seeds containing embryos grow into normal sprouts. These factors affect the viability of each combination and the ability to produce the normal sprouts that are expected to become new clones. A failure in the kenaf reproductive process could be the result of imperfect pollination. This means that fertilization does not occur, ovules do not develop, and seed endosperm does not develop normally. Consequently, the seeds do not germinate, and weakly growing sprouts are abnormal or die so that the sprouts do not grow into normal plants (Kuligowska et al. 2015, Martin. 1982).

CONCLUSION

There were several degrees of compatibility for the interspecific hybrids between eight parental genotypes of kenaf and three genotypes of its close relatives. The combination of hybrids were compatible (25 combinations), partly incompatible (7 combinations), incompatible (4 combinations), and very incompatible (18 combinations). The compatibility of interspecific hybrids between kenaf as a parental female or parental male and *H. radiatus* was greater than the compatibility of hybrids of kenaf with *H. acetocella* or *H. sabdariffa*. The viability of seed produced from the interspecific hybrids between kenaf and its close relatives was 0%. The viability of seed from reciprocal crosses between kenaf as parental male and *H. radiatus* was 41.5%, and between kenaf and *H. acetocella* was 31.5%. While the viability of seed from the reciprocal cross between kenaf and *H. sabdariffa* was 0%.

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Appendix

Table 3. Cross number, formed fruit, percentage of successful crosses, the number of seeds per fruits, compatibility type.

Interspecific hybrid (♀x♂)	Cross number	Fruit sets	Cross Success (%)	Seeds number/fruits	Compatibility
KR 1 x Kal II	37	17	45.95	137	Compatible
KR 1 x SSRH	44	5	11.36	24	Partially compatible
KR 1 x HS40	66	12	18.18	59	Partially compatible
KR 4 x Kal II	50	25	50	78	Compatible
KR 4 x SSRH	52	11	21.15	85	Partially compatible
KR 4 x HS40	45	5	11.11	57	Partially compatible
KR 5 x Kal II	39	30	76.92	118	Compatible
KR 5 x SSRH	52	14	26.92	99	Partially compatible
KR 5 x HS40	38	0	0	0	Very Incompatible
KR 6 x Kal II	66	52	78.79	95	Compatible
KR 6 x SSRH	74	3	4.05	27	Very Incompatible
KR 6 x HS40	56	3	5.36	25	Fully Incompatible
KR 15 x Kal II	51	29	56.86	111	Compatible
KR 15 x SSRH	47	9	19.15	91	Partially compatible
KR 15 x HS40	45	2	4.44	20	Very

					Incompatible
Kin 2 x Kal II	43	29	67.44	105	Compatible
Kin 2 x SSRH	53	10	18.87	55	Partially compatible
Kin 2 x HS40	49	3	6.12	30	Very Incompatible
DS028 x Kal II	83	73	87.95	94	Compatible
DS028 x SSRH	117	35	29.91	74	Compatible
DS028 x HS40	49	24	48.98	82	Fully incompatible
Cuba x Kal II	65	55	84.62	213	Compatible
Cuba x SSRH	54	6	10.5	87	Very Incompatible
Cuba x HS40	57	6	10.53	0	Fully incompatible
Reciprocal cross					
Kal II x KR 1	47	45	95.74	191	Compatible
Kal II x KR 4	41	39	95.12	183	Compatible
Kal II x KR 5	48	47	97.92	205	Compatible
Kal II x KR 6	47	46	97.87	196	Compatible
Kal II x KR 15	43	40	93.02	118	Compatible
Kal II x Kin 2	52	50	96.15	125	Compatible
Kal II x DS028	99	94	94.95	191	Compatible
Kal II x Cuba	52	18	34.62	254	Compatible
Kal II x SSRH	49	36	73.47	62	Fully incompatible
Kal II x HS40	55	0	0	0	Fully incompatible
SSRH x KR 1	79	36	45.57	140	Compatible
SSRH x KR 4	64	44	68.75	129	Compatible
SSRH x KR 5	72	50	69.44	163	Compatible

SSRH x KR 6	169	85	50.3	148	Compatible
SSRH x KR 15	74	24	32.43	163	Compatible
SSRH x Kin 2	72	62	86.11	153	Compatible
SSRH x DS028	88	54	61.36	165	Compatible
SSRH x Cuba	178	77	43.26	160	Compatible
SSRH x Kal II	76	33	43.42	137	Fully Incompatible
SSRH x HS40	75	0	0	0	Fully Incompatible
HS40 x KR 1	48	16	33.33	0	Fully Incompatible
HS40 x KR 4	43	13	30.23	0	Fully Incompatible
HS40 x KR 5	48	14	29.17	0	Fully Incompatible
HS40 x KR 6	55	5	9.09	0	Fully Incompatible
HS40 x KR 15	49	10	20.41	0	Fully Incompatible
HS40 x Kin 2	45	10	22.22	0	Fully Incompatible
HS40 x DS028	44	15	34.09	0	Fully Incompatible
HS40 x Cuba	47	4	8.51	0	Fully Incompatible
HS40 x Kal II	45	7	15.56	0	Fully Incompatible
HS40 x SSRH	48	0	0	0	Fully Incompatible

Self-pollination					
KR 1 x KR 1	66	66	100	195	Compatible
KR 4 x KR 4	48	48	100	217	Compatible
KR 5 x KR 5	36	34	94.44	258	Compatible
KR 6 x KR 6	65	65	100	206	Compatible
KR 15 x KR 15	59	50	84.75	266	Compatible
Kin 2 x Kin 2	50	45	90	254	Compatible
DS028 x DS028	189	172	91	206	Compatible
Cuba x Cuba	63	56	88.89	164	Compatible
Kal II x Kal II	57	51	89.47	133	Compatible
SSRH x SSRH	57	56	98.25	53	Compatible
HS40 x HS40	55	43	78.18	39.45	Compatible

Note:

KR : Karangploso

Kin 2 : Kenafindo 2 Agribun

Cuba : Cuba 108/Italia

DS028 : *H.cannabinus*

Kal II : *H. radiatus*

SSRH : *H. acetocella*

HS40 : *H. sabdariffa*

Table 4. The viability of seeds from the interspecific hybrids between *H.cannabinus* L. and its close relatives, reciprocal cross, and self-pollination.

Interspecific hybrid	Seeds number	Plant growth		Germination (%)	Reciprocal cross	Seeds number	Plant growth		Germination (%)
		Normal	abnormal				Normal	abnormal	
1	2	3	4	5	6	7	8	9	10
KR 1 x Kal II	50	0	50	0	Kal II x KR 1	50	22	28	44
KR 1 x SSRH	50	0	50	0	SSRH x KR 1	50	9	41	18
KR 1 x HS40	50	0	50	0	HS40 x KR 1	50	0	50	0
KR 4 x Kal II	50	0	50	0	Kal II x KR 4	50	26	24	52
KR 4 x SSRH	50	0	50	0	SSRH x KR 4	50	17	33	34
KR 4 x HS40	50	0	50	0	HS40 x KR 4	50	0	50	0
KR 5 x Kal II	50	0	50	0	Kal II x KR 5	50	18	32	36
KR 5 x SSRH	50	0	50	0	SSRH x KR 5	50	17	33	34
KR 5 x HS40	50	0	50	0	HS40 x KR 5	50	0	50	0
KR 6 x Kal II	50	0	50	0	Kal II x KR 6	50	30	20	60
KR 6 x SSRH	50	0	50	0	SSRH x KR 6	50	19	31	38

KR 6 x HS40	50	0	50	0	HS40 x KR 6	50	0	50	0
KR 15 x Kal II	50	0	50	0	Kal II x KR 15	50	21	29	42
KR 15 x SSRH	50	0	50	0	SSRH x KR 15	50	14	36	28
KR 15 x HS40	50	0	50	0	HS40 x KR 15	50	0	50	0
Kin 2 x Kal II	50	0	50	0	Kal II x Kin 2	50	15	35	30
Kin 2 x SSRH	50	0	50	0	SSRH x Kin 2	50	16	34	32
Kin 2 x HS40	50	0	50	0	HS40 x Kin 2	50	0	50	0
DS028 x Kal II	50	0	50	0	Kal II x DS028	50	15	35	44
DS028 x SSRH	50	0	50	0	SSRH x DS028	50	16	34	32
DS028 x HS40	50	0	50	0	HS40 x DS028	50	0	50	0
Cuba x Kal II	50	0	50	0	Kal II x Cuba	50	12	38	24
Cuba x SSRH	50	0	50	0	SSRH x Cuba	50	18	32	36
Cuba x HS40	50	0	50	0	HS40 x Cuba	50	0	50	0
Kal II x SSRH	50	22	28	44	SSRH x Kal II	50	31	19	0
Kal II x	50	0	50	0	HS40 x	50	0	50	0

HS40 SSRH x HS40	50	0	50	0	Kal II HS40 x SSRH	50	0	50	0

Self-pollination	Seeds number	Plant growth		Germination (%)
		Normal	abnormal	
KR 1 x KR 1	100	96	4	95.66
KR 4 x KR 4	100	95	5	95
KR 5 x KR 5	100	95	5	95.33
KR 6 x KR 6	100	98	2	97.66
KR 15 x KR 15	100	94	6	94
Kin 2 x Kin 2	100	96	4	96.33
DS028 x DS028	100	95	5	95
Cuba x Cuba	100	96	4	96
Kal II x Kal II	100	91	9	90.66
SSRH x SSRH	100	92	8	91.66
HS40 x HS40	100	96	4	96