Viability and vigor of sugarcane (Saccharum spp. Hybrid) seeds from intrageneric and intergeneric crosses at various storage periods

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Abstract. Sugarcane is one of the essential crops with high economic value in Indonesia, because it is the primary source of sugar. Sugarcane is propagated vegetatively for commercial purposes, but for genetic improvement, crosses carried out to produce seeds with the new genetic combinations. This study aims to determine the viability and vigor of seeds from intrageneric and intergeneric crosses at various storage periods. This study used seed samples from 19 intrageneric and intergeneric crosses conducted by ISRI from 2006 to 2016. The seeds were kept in aluminum foil bags in the freezer at -20°C. Viability and vigor of seeds were determined by evaluating germination, growth speed, growth synchronously, vigor index, and maximum growth potential. The results showed that the type of crosses and the storage time of seeds had a very significant effect on germination, growth speed, growth synchronously, vigor index, and maximum growth potential. However, the longer storage of sugarcane seeds was not always accompanied by a decrease in seed viability and vigor, both seeds resulting from intergeneric and intrageneric crosses. In addition, the viability and vigor of seeds from intergeneric crosses was lower than the viability and vigor of seeds from intrageneric crosses.

Keywords: breeding, germination, growth, parental

1. Introduction
Sugarcane (Saccharum spp.) is a crucial crop in the world as a sugar-producing plant. In addition, the economic value of sugarcane has also increased as a result of its ability to produce sustainable energy [1]. In Indonesia, sugarcane is one of the crucial industrial crops because sugar is considered a strategic food commodity. Sugar is a source of income for more than 750,000 farmers and nearly 250,000 workers directly involved in the sugarcane processing sector [2].

A sugarcane breeding program to assemble new superior varieties with the desired properties, should be carried out continuously to meet the needs of varieties for farmers and sugar industries. Sugarcane is a cross-pollinated plant and its commercial propagation is vegetatively. However, seed production from crosses is crucial for sugarcane genetic improvement programs. Genetic improvement was done by selecting the offspring population and cloning the superior offspring obtained from the crosses [3]. In sugarcane, the induction of flowering, crossing and seed production only occurs at a certain temperature and photoperiodicity. In the tropics, sugarcane flowering occurs naturally, while in sub-tropical and temperate regions, flowering is regulated using photoperiodicity facilities [4].
In the sugarcane genetic breeding programs, the production of seeds is performed using two types of breeding: bi-parental and multiple. In the bi-parental crossing, the hybridization is made between two genotypes of interest, while in the multiple crossing, only the identity of the mother plant is known, and the pollen come freely from different individuals [5]. The effects of these crossing methods over the formation of viable seeds and, or with higher physiological potential are unknown, which enhances the need for investigation. Single-seeded sugarcane is ovoid, yellowish-brown, and very small (about 1 mm long). The real sugarcane seed is caryopsis, which is formed from a single carpel, the ovule wall is fused with the seed coat. The collected seeds are called fuzz or ‘fluff’ [6].

Every year, the Indonesian Sugar Research Institute (ISRI) conducts a sugarcane breeding program by bi-parental crossing among sugarcane, or sugarcane with other genera to produce seeds selected as sugarcane breeding materials. After seed harvest, 1-2 grams of seeds from the crosses sown at that time, while the remaining seeds stored in a freezer at -20°C to provide seeds for the next season or for use in specific breeding programs. Until now, ISRI still keeps some seeds from crosses from 2006 to 2016, consisting of seeds from intrageneric crosses (between sugarcane and sugarcane) and intergeneric crosses (between sugarcane and Erianthus arundinaceus or between sugarcane and sweet sorghum). Prior to use in breeding programs, seeds were tested to determine their viability and vigor. Therefore, it was necessary to test the viability and vigor of sugarcane seeds from different types of crosses, both intergeneric and intrageneric crosses, stored at different storage periods.

2. Materials and methods
2.1. Studied sites
This research was conducted from May to September 2017 at the Indonesian Sugar Research Institute (ISRI) Pasuruan at an altitude of 0-4 meters above sea level. The average annual temperature was 27.4°C, and the average annual precipitation was 2,435 mm.

2.2. Materials
This study used seeds from 19 types of crosses representing intergeneric, intrageneric, interspecific and intraspecific crosses from 2006 to 2016, stored in a freezer at -20°C. In each crossing, 100 true seeds (caryopsis) were taken randomly for germination test with four replicates, for a total of 400 seeds per crossing. The seeds sown in plastic boxes containing sterilized media in a mixture of sand, soil, and P fertilizer.

2.3. Observation
- Observation of the germination (G) of sugarcane seeds in this study carried out two times [7]. Count I was carried out on 15 days after sowing, count II carried out on 24 days after sowing. The formula for G is:
  \[ G(\%) = \frac{\sum (\text{observation I + II})}{\text{the number of seeds sown}} \times 100\% \]

- Growth rate (GR) indicates growing strength vigor. GR was measured based on the percentage of normal germination when the seeds sown until the end of the observation (6 weeks after the seeds sown). Observations made every day, the percentage of addition of normal seedlings on the day of observation was divided by et mal (1 et mal=24 hours). The formula for GR is:
  \[ \text{GR(\%) = } \sum \frac{N_1}{D_1} + \frac{N_2}{D_2} + \ldots + \frac{N_e}{D_e} \]

  \( N_1,2,..e \): percentage of growth of normal germination at the time of observation
  \( D_1,2,..e \): observation time/number of days after planting (et mal)
  \( e \): the end of observation

- Growth synchronously (GS) was measured based on the percentage of normal germination on the day between germination observations in Count I and Count II. In this study, germination
was counted on the 15th and 24th days after the seeds sown, so that the growth synchronously calculated on the 19th day after sowing with the following formula:

\[
\text{GS} (%) = \frac{\text{the number of sprouts at the 19th}}{\text{the number of seeds sown}} \times 100\%
\]

- The vigor index (VI) was determined based on the percentage of the number of seeds that normally germinated at the first count observation. Vigor index calculated from the percentage of normal germination on the first day of observation divided by the total germinated seeds with the following formula:

\[
\text{VI} (%) = \frac{\text{the number of sprouts at the first of the observation}}{\text{the number of seeds sown}} \times 100\%
\]

- Maximum Growth Potential (MGP) is a measure of total viability that shows the ability of a seed to survive. The maximum growth potential was calculated based on the number of seeds that grew, both normal and abnormal, on the last observation day, six weeks after sowing with the following formula:

\[
\text{MGP} (%) = \frac{\text{the number of sprouts at the end of the observation}}{\text{the number of seeds sown}} \times 100\%
\]

- After the seedlings were approximately six weeks after sowing, they were transferred to polybags to strengthen their roots before planting them in the field.

2.4. Experimental and statistical analysis
The experiment designed using a completely randomized design (CRD) with 19 treatments, as shown in Table 1. This experiment was carried out with four replications so that there were 76 experimental units. The sugarcane seeds used in each replication were 100 seeds, so the whole seeds used in this study were 7,600 seeds. Sugarcane seeds that germinated and survived up to six weeks after sowing from each treatment transferred to polybags.

### Table 1. Type, combination, and year of the hybridizations to produce seeds used in this study

| Types of hybridizations                  | Species/genera                | Treatment codes | Crossing parents combinations | Crossing year |
|-----------------------------------------|-------------------------------|-----------------|-------------------------------|---------------|
| Intergeneric hybridization              | *Saccharum* spp.              |                 |                               |               |
|                                        | Hybrid x                      |                 |                               |               |
|                                        | *Erianthus arundinaceus*      | P1              | PS 862 X IJ 76-378            | 2011          |
|                                        |                               | P2              | PS 862 X IJ 76-374            | 2013          |
| Intergeneric hybridization              | *Saccharum* spp.              |                 |                               |               |
|                                        | Hybrid x                      |                 |                               |               |
|                                        | *Sorghum bicolor*             | P3              | M 442-51 X Kawali             | 2013          |
|                                        |                               | P4              | BL X Kawali                   | 2014          |
| Intrageneric/Interspecific hybridization| *Saccharum* spp.              |                 |                               |               |
|                                        | Hybrid x                      |                 |                               |               |
|                                        | *Saccharum* spp.              | P5              | D 9388 X BU 442               | 2006          |
|                                        | Hybrid x                      | P6              | CP 52-48 X PS 864             | 2007          |
|                                        |                               | P7              | RB 72-5042 X PS 41            | 2008          |
|                                        |                               | P8              | KK X PS 80-1993               | 2009          |
|                                        |                               | P9              | TROJAN X PS 94-516            | 2013          |
|                                        |                               | P10             | BL X F 156                    | 2014          |
|                                        |                               | P11             | TUC 72-5 X CP 82-1592         | 2015          |
|                                        |                               | P12             | CP 75-1082 X PS 71-586        | 2016          |
| Intrageneric/Interspecific hybridization| *Saccharum* spp.              |                 |                               |               |
|                                        | Hybrid x                      |                 |                               |               |
|                                        | *Saccharum officinarum*       | P13             | POI 2878 X IJ 76-518          | 2014          |
|                                        |                               | P14             | TUC 72-5 X IJ 76-348          | 2015          |
|                                        |                               | P15             | CP 29-116 X IJ 76-318         | 2016          |
| Intrageneric/Interspecific hybridization| *Saccharum* spp.              |                 |                               |               |
|                                        | Hybrid x                      |                 |                               |               |
|                                        | *Saccharum officinarum*       | P16             | IJ 76-450 X PS 57             | 2006          |
|                                        |                               | P17             | IS 76-200 X CP 70-1133        | 2007          |
|                                        |                               | P18             | IJ 76-465 X CP 82-1592        | 2015          |
|                                        |                               | P19             | IJ 76-518 X CP 44-101         | 2016          |
The data in this study were analyzed using analysis of variance (ANOVA) or F test. If the analysis of variance showed significantly different effects, further Tukey’s test and orthogonal contrast were

| No | Treatment groups | Types and years of hybridizations | No | Treatment groups | Types and years of hybridizations |
|----|------------------|----------------------------------|----|------------------|----------------------------------|
| 1  | P1,P2,P3,P4 vs. P5,P6,P7,P8,P9, P10,P11,P12,P13,P14,P15,P16, P17,P18, P19 | Intergeneric hybridizations (2006, 2007, 2008, 2009, 2013, 2014, 2015, 2016) | 10 | P4 vs. P10 | Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2014) vs. Intrageneric hybridizations Saccharum spp. Hybrid x Saccharum spp. Hybrid (2014) |
| 2  | P1,P2 vs. P3,P4 | Intergeneric hybridizations (Saccharum spp. Hybrid x E. arundinaceus) (2011, 2013) vs. Intergeneric hybridizations (Saccharum spp. Hybrid x Sorghum bicolor) (2015, 2014) | 11 | P4 vs. P13 | Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2014) vs. Intergeneric hybridization Saccharum spp. Hybrid x S. officinarum (2014) |
| 3  | P5,P6,P7,P8,P9, P10,P11, P12 vs. P13,P14,P15,P16,P17,P18,P19 | Intergeneric/intraspecific hybridizations (Saccharum spp. Hybrid x Sorghum bicolor) (2006, 2007, 2008, 2009, 2013, 2014, 2015, 2016) vs. Interspecific hybridizations (Saccharum spp. Hybrid x S. officinarum x Saccharum spp. Hybrid) (2006, 2007, 2014, 2015, 2016) | 12 | P10 vs. 13 | Intergeneric hybridization Saccharum spp. Hybrid x Sorghum spp. Hybrid (2014) vs. Interspecific hybridization Saccharum spp. Hybrid x S. officinarum (2014) |
| 4  | P13,P14,P15 vs. P16,P17,P18,P19 | Interspecific hybridizations (Saccharum spp. Hybrid x S. officinarum) (2014, 2015, 2016) vs. Interspecific hybridizations (S. officinarum x Saccharum spp. Hybrid) (2006, 2007, 2015, 2016) | 13 | P11 vs. P14 | Intergeneric hybridization Saccharum spp. Hybrid x Sorghum bicolor (2015) vs. Interspecific hybridizations Saccharum spp. Hybrid x S. officinarum (2015) |
| 5  | P2 vs. P3 | Intergeneric hybridizations Saccharum spp. Hybrid x E. arundinaceus (2013) vs. Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2013) | 14 | P11 vs. P18 | Intergeneric hybridization Saccharum spp. Hybrid x Sorghum bicolor (2015) vs. Interspecific hybridizations S. officinarum x Saccharum spp. Hybrid (2015) |
| 6  | P2 vs. P9 | Intergeneric hybridizations Saccharum spp. Hybrid x E. arundinaceus (2013) vs. Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2013) | 15 | P14 vs. P18 | Intergeneric hybridizations Saccharum spp. Hybrid x S. officinarum (2015) vs. Interspecific hybridizations S. officinarum x Saccharum spp. Hybrid (2015) |
| 7  | P3 vs. P9 | Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2013) vs. Intergeneric hybridizations Saccharum spp. Hybrid x Saccharum spp. Hybrid (2013) | 16 | P12 vs. P15 | Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2016) vs. Interspecific hybridizations Saccharum spp. Hybrid x S. officinarum (2016) |
| 8  | P5 vs. P16 | Intergeneric hybridizations Saccharum spp. Hybrid x Saccharum spp. Hybrid (2006) vs. Interspecific hybridizations S. officinarum x Saccharum spp. Hybrid (2006) | 17 | P12 vs. P19 | Intergeneric hybridizations Saccharum spp. Hybrid x Sorghum bicolor (2016) vs. Interspecific hybridizations S. officinarum x Saccharum spp. Hybrid (2016) |
| 9  | P6 vs. P17 | Intergeneric hybridizations Saccharum spp. Hybrid x Saccharum spp. Hybrid (2007) vs. Interspecific hybridizations S. officinarum x Saccharum spp. Hybrid (2007) | 18 | P15 vs. P19 | Intergeneric hybridization Saccharum spp. Hybrid x S. officinarum (2016) vs. Interspecific hybridization Saccharum spp. Hybrid x S. officinarum (2016) |
performed at the 5% and 1% levels. Orthogonal contrast was used to compare groups of different hybridization types; between genera, and within genera, between species, and within species, in the same or different years of crossing, as shown in Table 2. Types of hybridization include intragenic, interspecific, and intergeneric crosses. The year of hybridization indicates the storage period of the analyzed seeds.

3. Results and discussions

3.1. Viability and vigor of sugarcane seeds
Sugarcane is propagated vegetatively using cuttings and seeds from crosses are not used in commercial seed propagation. In 1858, sugarcane's ability to produce seeds was discovered in Java, then re-observed in Barbados in 1859, making it possible to cross sugarcane [8]. Knowledge about sexual reproduction in sugarcane is still very limited, because sugarcane is propagated vegetatively, so most research is focused on genetic improvement.

Seed cane or “fuzz” is the whole panicle cane without the central axis and lateral axis greater. Thus, mature “fuzz” consists of mature fruit (caryopsis), glumes, callus hairs, old anthers and stigmas, and pieces of rachis. These parts of the inflorescence are generally handled, stored, and sown with seeds because it is impractical to separate the small, bare seeds without damaging them. The coils of callus hairs (long bristles) surrounding each sugarcane's tiny flower give it a 'blurry' look, hence the name cane ‘fuzz’ or ‘fluff’. This heterogeneous mixture of flower parts (fuzz) is what breeders treat as the 'true seed' of sugarcane [5].

This study involved seeds from different types of hybridization and crossing years. Types of crosses include intragenic, interspecific, and intergeneric crosses. Intragenic crosses between *Saccharum* spp. Hybrid and *Saccharum* spp. Hybrid, interspecific crosses between *Saccharum* spp. Hybrid and *Saccharum officinarum*, where *S. officinarum* was the donor and recipient of pollen, and intergeneric crosses between *Saccharum* spp. hybrid and *Erianthus arundinaceus* and *Saccharum* spp. Hybrid with *Sorghum bicolor*. Meanwhile, these crosses were performed in different years, between 2006 and 2016. The seeds from 19 different types and years crosses were tested for their viability and vigor.

The analysis of variance on the effect of treatment types and years of crossing on viability and vigor of sugarcane seeds in nineteen crosses analyzed are shown in Table 3. Table 3 shows that the types and years of the crosses had a very significant effect on germination, growth rate, growth synchronously, index of vigor, and maximum growth potential of the seeds analyzed according to the F test at the 1% probability level.

| Parameters                  | F Crit | cv (%) |
|-----------------------------|--------|--------|
| Germination (G)             | 35.26**| 0.22   |
| Growth rate (GR)            | 37.30**| 0.22   |
| Growth synchronously (GS)   | 18.45**| 0.31   |
| Vigor Index (VI)            | 28.59**| 0.25   |
| Maximum growth potential (MGP)| 20.46**| 0.29   |

F 5% = 1.79  
F 1% = 2.77

Notes: ** = Very significantly different according to the F test at the 1% probability level.

The results of the Tukey’s test on the effect of treatment on all viability and vigor parameters of sugarcane seeds analyzed are shown in Table 4. Meanwhile, comparisons on the average viability and vigor parameters of sugarcane seeds of various types and years of crossing using orthogonal contrast are shown in Table 5.

According to Tukey's test at 1% level, seeds germination and growth rate of treatments P8, P11, P12, and P18 were significantly different from seeds germination and growth rate from the other 15
treatments. Meanwhile, the parameters of seeds growth synchronously, vigor index, and maximum growth potential of P8, P12, and P18 were significantly different from seeds germination and growth rate of the other 16 treatments, as shown in Table 4. Based on the results of this study, the type of cross affected the viability and vigor of the seeds produced.

**Table 4.** Percentage of germination (G), growth rate (GR), growth synchronously (GS), vigor index (VI), maximum growth potential (MGP) from different hybridizations among *S. hybrid, E. arundinaceus, S. officinarum* and sweet sorghum in different cross year

| Treatments                              | G (%) | GR (%) | GS (%) | VI (%) | MGP (%) |
|-----------------------------------------|-------|--------|--------|--------|---------|
| P1 (PS 862 x E. arundinaceus II 76-378, 2011) | 0.50 a | 0.98 ab | 0.50 a | 0.25 a | 0.25 a |
| P2 (PS 862 x E. arundinaceus II 76-374, 2013) | 1.50 ab | 1.76 ab | 0.25 a | 0.75 a | 0.75 a |
| P3 (M 442-51 x Sweet Sorghum, 2013) | 1.20 ab | 1.80 a | 0.25 a | 0.75 a | 0.50 a |
| P4 (BL x Sweet Sorghum, 2014) | 0.50 a | 0.54 a | 0.00 a | 0.25 a | 0.25 a |
| P5 (D9388 x BRU442, 2006) | 1.00 ab | 1.22 ab | 0.25 a | 0.50 a | 0.50 a |
| P6 (CP 52-48 x PS 864, 2007) | 0.50 a | 0.92 a | 0.00 a | 0.50 a | 0.00 a |
| P7 (RB 72-5042 x PS 41, 2008) | 0.25 a | 0.46 ab | 0.50 a | 0.00 a | 0.25 a |
| P8 (K x PS 80-1993, 2009) | 19.00 ab | 27.53 ab | 9.25 ab | 9.75 ab | 9.25 ab |
| P9 (Trojan x PS 94-516, 2013) | 0.75 ab | 1.21 ab | 0.50 a | 0.25 a | 0.50 a |
| P10 (BL x F156, 2014) | 1.00 ab | 2.02 ab | 0.25 a | 1.00 ab | 0.00 a |
| P11 (TUC 72-5 x CP 82-1592, 2015) | 5.00 b | 7.32 b | 2.00 a | 2.75 a | 2.25 a |
| P12 (CP 75-1082 x PS 71-5856, 2016) | 15.75 a | 23.47 c | 7.75 b | 8.25 b | 7.50 b |
| P13 (POG 2878 x S. officinarum II 76-518, 2014) | 0.50 a | 1.17 a | 0.25 a | 0.50 a | 0.00 a |
| P14 (TUC 72-5 x S. officinarum II 76-348, 2015) | 1.00 ab | 0.87 a | 0.25 a | 0.25 a | 0.75 a |
| P15 (CP 29-116 x S. officinarum II 76-518, 2016) | 0.50 a | 0.51 a | 0.00 a | 0.25 a | 0.25 a |
| P16 (S. officinarum II 76-450 x PS 57, 2006) | 0.75 ab | 1.19 ab | 0.25 a | 0.50 a | 0.25 a |
| P17 (S. officinarum II 76-200 x CP 70-1133, 2007) | 2.50 ab | 2.49 ab | 2.25 a | 0.00 a | 2.50 a |
| P18 (S. officinarum II 76-465 x CP 82-1592, 2015) | 23.75 a | 34.96 b | 11.00 b | 12.25 b | 11.50 b |
| P19 (S. officinarum II 76-518 x CP44-101, 2016) | 0.25 a | 0.27 a | 0.25 ab | 0.00 a | 0.25 a |

Notes: The mean value followed by the same small letter in the same column, shows no difference according to Tukey’s test at the 5% probability level.

Among the 19 different types of hybridization in this study, there were three types of hybridizations had seeds with high viability and vigor, namely P8, P12 and P18. P18 had the highest values on germination, growth speed, growth synchronously, vigor index, and maximum growth potential (Figure 1). The three types of seeds were seeds resulting from intrageneric crosses between *S. hybrid* x *S. hybrid* (P8: Kidang Kencana x PS 80-1993 in 2009, P12: CP 75-1082 (introduced from Canal Point) x PS 71-586 in 2016), while P18 was an interspecific hybridization between *S. officinarum* (II 76-465) x CP 82-1592 in 2015. On the other hand, low seed viability and vigor occurred in seeds from crosses between genera, namely *Saccharum* spp. Hybrid x sweet sorghum and *Saccharum* spp. Hybrid x *E. arundinaceus*.

The effect of treatments on seed germination in this study showed that the percentage of sugarcane seed germination was between 0.25% - 23.75%. Germination in this study was observed twice, namely 15 days after sowing and 24 days after sowing. The highest germination was at P18 of 23.75%, while the lowest was at P7 and P19 of 0.25%. P18 is an interspecific hybridization between *Saccharum Officinarum X Saccharum* spp. Hybrid in 2015, while P7 is an intrageneric hybridization between *Saccharum* spp. Hybrid X *Saccharum* spp. Hybrid in 2008, and P19 is an interspecies crossing of *Saccharum officinarum x Saccharum* spp. Hybrid in 2016. Based on these results, it shows that the percentage of sugarcane seed germination is low. This is in line with the results of research conducted by [3], [9], which stated that the germination of sugarcane seeds was low. In addition, [10] reported that the percentage of germination in sugarcane seeds from crosses was low and the maximum was only 49%. Sugarcane is a species that produces few seeds and has low viability. Because sugarcane is different from other types of cereals. In other cereals the expected part is the seed, while sugarcane is expected to be sugar stored in the stem, so that flowering in sugar cane is not desired.
Seed vigor observed were storage vigor, growth vigor, and genetic vigor. Simultaneous growth shows the vigor of storage capability, because simultaneous growth shows the relationship with storage capability. High synchronous growth indicates high seed storage ability as well. In comparison, the speed of growth is one of the measures of the vigor parameter of growth strength. The growth speed is closely related to seed vigor. Seeds with high growth speed, the resulting plants tend to be more resistant to sub-optimum environmental conditions. Based on the results of this study, simultaneous growth and the highest growth rate were in treatment P18, followed by P8, and P12. Seeds that have a high rate of growth and simultaneously have a high vigor [7]. According to [11], simultaneous and speed of seed germination is an critical component of seed performance, and directly affects its vigor. Vigorous seeds more efficient in transmitting food reserves from storage sources to the embryonic axis. This ability is expressed in the strength of growth in seedlings. Therefore, in this study, the performance of seeds from these three types of crosses was better than the performance of seeds from other crosses.

Quantitative estimation of seed fertility is very crucial for sugarcane breeding as described by [6] as follows: "arrow" inflorescence consists of 25,000 spikelets, but the number of fertilized and fertile is only about 3-33%, which is about 700 spikelets that will the development become seeds. [12] reported that sugarcane seeds (caryopses) is very tiny (1.8 mm long and 0.8 mm wide), ellipsoid-shaped and brown. Internally, sugarcane seeds have a structure similar to seeds from members of the Poaceae family, which consists of a large endosperm covered by an aleurone layer with an embryo at its base. The embryo is relatively large, comprising a third of the volume of seed [6].

Seedling vigor depends on the genotype crossed. This can be seen from the results of this study. Seeds produced from interspecific hybridization between sugarcane and S. officinarum, in which S. officinarum as a pollen donor and recipient in the same crossing year showed differences in seed quality. Seeds from P19 had lower germination rate and vigor index than germination and seed vigor index at P15, in which P19 and P15 were interspecific crosses between sugarcane and S. officinarum in 2016. At P19, S. officinarum as the pollen recipient, while at P15, S. officinarum as a pollen donor. In contrast, seeds from P18 had the highest value on all viability and vigor indicators than seeds viability and vigor from P14, in which P18 and P14 were interspecific crosses in 2015. In P18, S. officinarum as the pollen recipient, while at P14, S. officinarum as a pollen donor. [13] reported that the RB92579 variety as a pollen donor and recipient had an effect on the viability and vigor of the seeds produced. However, contrary to the results of this study, hybridized seedlings involving RB92579 as a pollen donor showed...
stronger root growth compared to those produced from RB92579 as a pollen recipient in the same crossing year.

Interspecific hybridization is a way of introducing agronomically important genes from wild relatives for plant improvement programs. However, reproductive barriers often occur in hybridization between so different distant relatives and plant species that make introgression difficult. These barriers include lack of fertilization, endosperm failure, embryo abortion, seed killing, hybrid sterility, and hybrid damage. Usually, the common prezygotic reproductive barrier results from pollen-pistil incompatibility, so that pollen tube growth from one species is inhibited in the stigma of another species [14]. Therefore, low viability and vigor often occur in seeds from intergeneric and interspecific crosses as has been reported by several researchers. [15] reported that out of 96 trials of crosses between *Saccharum* and *Erianthus* only 26 were successful and produced 1000 seedlings. Thirty-seven out of the 1000 seedlings identified as hybrids, but only 19 seedlings survived. All surviving seedlings were known to have very low vigor levels. The results of this study are strengthened by the research conducted by [16]. She reported that the viability of sugarcane seeds from crosses between *Saccharum* spp. Hybrid *x* *Erianthus arundinaceus* ranged from 0.05 %-0.59%, while the viability of seeds from crosses between *Saccharum* spp. Hybrid *x* *Saccharum* spp. Hybrids were about 7.03%. This showed the viability of sugarcane seeds from intergeneric crosses was lower than the viability of seeds from intrageneric crosses.

Meanwhile, this study showed that the longer seed storage was not always followed by a decrease in seed viability, both seeds from intergeneric and intrageneric crosses. The storage period of seeds was indicated by the crossing year. Of the 19 hybridization types, there were seven types of crosses whose seedlings could survive until they were transferred to polybags at six weeks after sowing. The seven types of crosses were carried out in different years, namely P2 (intergeneric hybridization between *Saccharum* spp. Hybrid *x* *Erianthus arundinaceus* in 2013), P3 (intergeneric hybridization between *Saccharum* spp. Hybrid *x* *Sorghum bicolor* in 2013), P8, P10, P11, P12 (intrageneric hybridization between *Saccharum* spp. Hybrid *x* *Saccharum* spp. Hybrid in 2009, 2014, 2015, 2016, respectively), and P18 (interspecific hybridization between *S. officinarum* *x* *Saccharum* spp. Hybrid in 2016). These seven crosses could produced seeds with better growth vigor than seed vigor from other types of crosses.

3.2. Comparison of treatment groups of various types and years of the cross on viability and vigor of the seeds produced

Based on the results of the further orthogonal contrast test (Table 5), namely the comparison of viability and vigor including germination, growth speed, growth synchronously, vigor index, and maximum growth potential between sugarcane seeds from intrageneric and intergeneric crosses in different crossing years, did not show significant differences. Likewise, the comparison of viability and vigor, including germination, growth speed, growth simultaneously, vigor index, and maximum growth potential between sugarcane seeds from intrageneric and intergeneric crosses in the same crossing year, also did not show significant differences.

Sugarcane seeds from crosses must be stored in the right conditions because crossing is not an easy job. According to [6], seeds can be stored on aluminum foil in the freezer at a temperature of approximately -20°C and a humidity of 15-25%. The seeds quickly lose their viability, but if stored at low temperatures, they can be maintained for at least three years[6], while [17] reported that the conservation of viability of sugarcane seeds when stored at -20°C, the germination potential of seeds could be maintained for up to five years.

[18, 10] reported that temperature was not a limiting factor for sugarcane germination. Sugarcane seeds can germinate at a temperature of 11°C-42°C, but sugarcane has an optimum temperature for germination, in the range of 27°C-36°C [18]. [10] reported significant differences in the yield of seed germination at various temperatures. The appearance and health of the seedling are better at a temperature of 30°C-39°C. Availability of water is a critical factor in sugarcane seed germination. Therefore, the water availability at the time of seed germination needs to be taken into account to be evaluated. Low water potential will slow down the germination process due to the increased difficulty of the seeds to absorb water from the surroundings. The intensity of the lack of water, will inhibit or
greatly assist germination depending on the species. Seedling media is also one of the environmental factors in sugarcane seed germination.

[19] stated that seeds of 13 sugarcane genotypes stored in laboratory conditions at 22°C for 10-12 weeks, more than half of them still have viable seeds. Sugarcane seeds that are simply placed at 28°C lose 90% of their viability within 70 days. The treatment during storage is very influential on the deterioration of seed viability. Physiological potential and seed life span are related to genetic characteristics and storage time. According to [6], sugarcane seeds lose their viability immediately, but the seeds can maintain their viability for a long time if frozen. If fresh seeds will be planted within two weeks, they should be stored in a desiccator. [17] stated that conservation of viability of sugarcane seeds when stored at a temperature of -20°C can maintain seed viability within five years. In this study, seeds with high viability and vigor were produced from crosses made for less than five years, except for P8 which was a 2009 cross. This indicates that treatment during storage is a critical factor that can affect seed viability and vigor of sugarcane seeds.

Table 5. Comparison of type and year of crosses using orthogonal contrast test on germination (G), growth rate (GR), growth synchronously (GS), vigor index (VI) and maximum growth potential (MGP)

| Comparable treatments          | Parameters | G (%)     | GR (%) etmal  | GS (%)     | VI (%)     | MGP (%)   |
|--------------------------------|------------|-----------|---------------|------------|------------|-----------|
|                                |            |           |               |            |            |           |
| P1,P2,P3,P4 vs. P5,P6,P7,P8,P9,P10,P11,P12,P13,P14,P15,P16,P17,P18,P19 |            | 0.548651* | 0.5392*       | 0.390817*  | 0.39268*   | 0.342877* |
| P1,P2 vs. P3,P4 vs. P5,P6,P7,P8,P9,P10,P11,P12 |            | 0.00013*  | 0.0018*       | 0.007426*  | 0.000257*  | 0.000976* |
|                                |            | 0.19162*  | 0.2589*       | 0.062554*  | 0.263117*  | 0.033353* |
|                                |            | 0.69486*  | 0.7083*       | 0.509363*  | 0.418415*  | 0.535169* |
|                                |            | 0.00025*  | 0.0001*       | 0*         | 0*         | 0*         |
|                                |            | 0.01009*  | 0.004*        | 0.003713*  | 0.015091*  | 0.001953* |
|                                |            | 0.000714* | 0.0055*       | 0.003713*  | 0.021173*  | 0*         |
|                                |            | 0.00178*  | 0.0002*       | 2.73298*   | 0*         | 0.004119* |
|                                |            | 0.03247*  | 0.0014*       | 0.102979*  | 0.021173*  | 0.161738* |
|                                |            | 0.00509*  | 0.0221*       | 0.003713*  | 0.03826*   | 0.004119* |
|                                |            | 0.00017*  | 0.0022*       | 0.003713*  | 0.002509*  | 0.004119* |
|                                |            | 0.00174*  | 0.0101*       | 0*         | 0*         | 0*         |
|                                |            | 0.18081*  | 0.2272*       | 0.862152*  | 0.20951*   | 0.058718* |
|                                |            | 1.44731*  | 1.6607*       | 1.44131*   | 1.164622*  | 0.9036*   |
|                                |            | 2.64639*  | 3.1164*       | 1.024132*  | 2.362059*  | 1.423003* |
|                                |            | 1.77861*  | 1.9328*       | 0.904512*  | 1.395198*  | 0.969079* |
|                                |            | 1.89305*  | 2.0107*       | 0.003713*  | 1.572366*  | 0.969079* |
|                                |            | 0.08178*  | 0.0008*       | 0.862152*  | 0.005293*  | 0*         |

Notes: ns = no significantly different according to the F test at the 5% probability level.

4. Conclusion
The longer the storage period of sugarcane seeds was not always followed by a decrease in the viability of sugarcane seeds in each type of cross, both intrageneric and intergeneric crosses. Viability and vigor of the seeds were influenced by the genotype of the parents and the years of the cross. The viability and vigor of seeds from intergeneric crosses were lower than seeds from intragenic crosses. Seeds with high viability and vigor were produced from crosses made for less than five years, if they were stored in freezer at -20°C.

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