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# ASSEMBLY OF SOYBEAN GENOTYPES DEVELOPED THROUGH THREE-WAY CROSS

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## SUMMARY

A three-way cross is an artificial cross performed to assemble superior cultivars for increased soybean production. The presented study aims to evaluate the percentage of success in the single cross and three-way cross of soybean genotypes to enhance the genetic variability in the base population of the genetic material. The genetic material comprises three soybean cultivars, i.e., Grobogan, Anjasmoro, and Dega-1. The three-way cross design operation made various crosses. Parameters observed were plant morphology, percentage of cross success and seed formation in pods, number of seeds in pod, and the total seed weight. The results showed a relatively high (57.14%) success rate for the cross Grobogan × Anjasmoro, producing numerous one-seeded and two-seeded pods and 30 seeds per plant with a total weight of 5.4 g. However, the success rate in three-way cross (F1 descent [Grobogan × Anjasmoro] × Dega-1) was medium (35.37%). The results of crossing the three genotypes of parents will greatly determine soybean variability.

**Keywords:** base population, genetic variability, single and three-way crosses, soybean, superior cultivars

**Key findings:** The three-way cross is a crossing technique to increase genetic diversity. High genetic diversity will benefit the creation of new cultivars with the desired characteristics. The success of the three-way cross will ensure to increase soybean production.

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**INTRODUCTION**

Soybean (*Glycine max* L. Merrill) is an annual legume belonging to the family Fabaceae, native to East Asia, and widely grown for its edible bean (Sundari and Pratiwi, 2018). Soybean is mostly an economically valuable dicot group plant globally because of its high protein and oil content. It provides vegetable protein for millions of people and ingredients for hundreds of chemical products (Rezyawati *et al*., 2018; Sulastri *et al*., 2020; Lestari *et al*., 2022). Soybeans contain significant amounts of phytic acid, dietary minerals, and B vitamins.

With unsuitable climatic conditions, soybean production decreases, probably due to low-yielding genotypes, inadequate growing areas, and inappropriate soil conditions (Chakma *et al.,* 2015; Nico *et al*., 2015; Adie and Krisnawati, 2019). National soybean production significantly declined, amounting to 632,300 t in 2020, then lowered to 613,300 and 594,600 t in 2021 and 2022, respectively (Agricultural Data Center and Information System, 2022).

Genetic diversity provides plant breeders to develop new and improved cultivars with desirable characteristics, including farmer-preferred traits with yield potential and large seeds. Genetic diversity facilitates breeders to develop cultivars for specific features like quality improvement and tolerance to biotic and abiotic stresses (Bhartiya *et al*., 2012; Bhandari *et al*., 2017; Mustofa *et al*., 2020). One of the efforts to increase soybean production is creating new variants through artificial crosses involving various superior parental genotypes. Artificial crosses aim to expand genetic diversity and combine the desired characters in one genotype. A variety that occurs naturally comes from the adaptation and domestication of each individual to the existing environment. Environmental factors also influence the visible nature (phenotype) of an individual, in addition to being determined by genetic factors (genotype). However, genetic diversity can also expand through artificial cross-breeding by involving various genotypes (Mulyaningsih and Indrayani, 2014).

Some factors determine the success of artificial crosses, which consists of a combination and compatibility between the distinct genotypes, flowering time, finesse of the breeders, as well as, the weather at the time of crossing. The weather conditions during crossing are essential in influencing the success rate of artificial crosses. The entwining should proceed when the air is chilly and the temperature ranges from 24 °C to 28 °C. When the air is hot, the pollen and pistil dry quickly; however, when the air is cold, the pollen will not become powdery (Adsul *et al*., 2016; Rasyad *et al*., 2018; Fatimah *et al.,* 2019).

Forming a base population with moderately high genetic diversity is the first step in assembling new cultivars. Base population formation can result from hybridization and mutation breeding among the genotypes with diverse genetic backgrounds. Artificial hybridization and crosses generate new variants with higher genetic diversity and further combine and acquire the desirable traits of various parent cultivars into a new and single genotype. Combining of these good traits can come from a three-way cross, i.e., (a × b) × c (Perez *et al.,* 2009; Arsyad *et al.,* 2016).

Soybean populations developed by three-way, four-way, and backcrosses have served the objective of new genotypes enhancement for yield increase. Past studies reported that in single and three-way cross comparison, three-way cross-population means were significantly higher than single crosses for yield, seed protein, and oil contents in soybean (Thorne and Fehr, 1970). Soybean lines obtained from single and three-way crosses and backcross populations revealed that populations developed by backcrossing had the highest seed yield, followed by three-way and single-cross populaces (Cianzio and Voss, 1994). Cober and Voldeng (2000) evaluated soybean lines from backcross and single-cross populaces and found that the average seed yield and protein content of backcross and single-cross lines differ nonsignificantly. Based on the above description, the concerned study aimed to assess the genetic potential of new variants obtained through three-way crosses in soybean.

## MATERIALS AND METHODS

### Plant material and procedure

The research transpired in a plastic house on USU's field, Universitas Sumatera Utara, Medan, Indonesia. The genetic material used in this study comprised three soybean cultivars, i.e., Grobogan, Anjasmoro, Dega-1, and their first $F_1$ hybrid (Grobogan × Anjasmoro) and second $F_1$ hybrid (F1 × Dega-1). The three-way cross design employed making various crosses. Before the study began, setting up a plastic house sized 8 m × 12 m occurred. Plant media used was in the form of topsoil mixed with compost, with a ratio of 5:1. Planting ran in different stages, i.e., cultivar Anjasmoro first, followed by cultivars Grobogan and Dega-1 after the trifoliate leaves appeared on cultivar Anjasmoro. Artificial crosses included castration, emasculation, pollination, isolation, and labeling.

### Parameters studied

In all the soybean populations, the data recording included the following three parameters: Plant morphology studied by observing the morphological characteristics of soybean; the success of the crosses followed by counting the number of flowers that undergo fertilization after being crossed.

$$\text{Success (\%)} = \frac{\text{Number of flowers that underwent fertilization}}{\text{Total number of crossed}} x\, 100\%$$

Acquiring the data on the number of seeds per pod came from counting the number of kernels in the pods.

## RESULTS AND DISCUSSION

### Plant morphology

Various morphological traits of three soybean cultivars, i.e., Grobogan, Anjasmoro, and Dega-1, are in Figure 1, with the mean values of the morphological parameters provided in Table 1. Based on observations, cultivar Dega-1 has the advantage of having taller plants and enhanced plant height compared with the two cultivars, Grobogan and Anjasmoro. For the number of productive pods, cultivar Dega-1 has 35.40 effective pods from the total number of pods (37.80). It indicates that cultivar Dega-1 produced more productive pods and had fewer empty ones than cultivars Grobogan and Anjasmoro because the difference between the number of abundant and total number of pods was only 2.4. In contrast, the differences between Grobogan and Anjasmoro were 20.53 and 4.36, respectively (Table 1).

The soybean cultivars revealed significant differences, with cultivar Dega-1 showing the maximum plant height, followed by Anjasmoro and Grobogan. Rather than shorter ones, the cultivars with taller plants benefit from receiving more sunlight throughout the canopy; hence, enhanced photosynthesis eventually boost the pod filling. The least plant height impacts the number of branches, productive nodes, and filled pods (Handriawan *et al.,* 2017). Plant height and the number of branches indicated positive correlation with the number of productive nodes, filled pods, and seed yield per plant (Hakim, 2017; Adie and Krisnawati, 2019).

In the presented study, several factors also influenced and managed the differences in the soybean cultivars for the number of branches per plant. According to Wang *et al*. (2013), the plant growth and number of branches gained impact from the genotypes, field practices, and inputs used. In soybean,

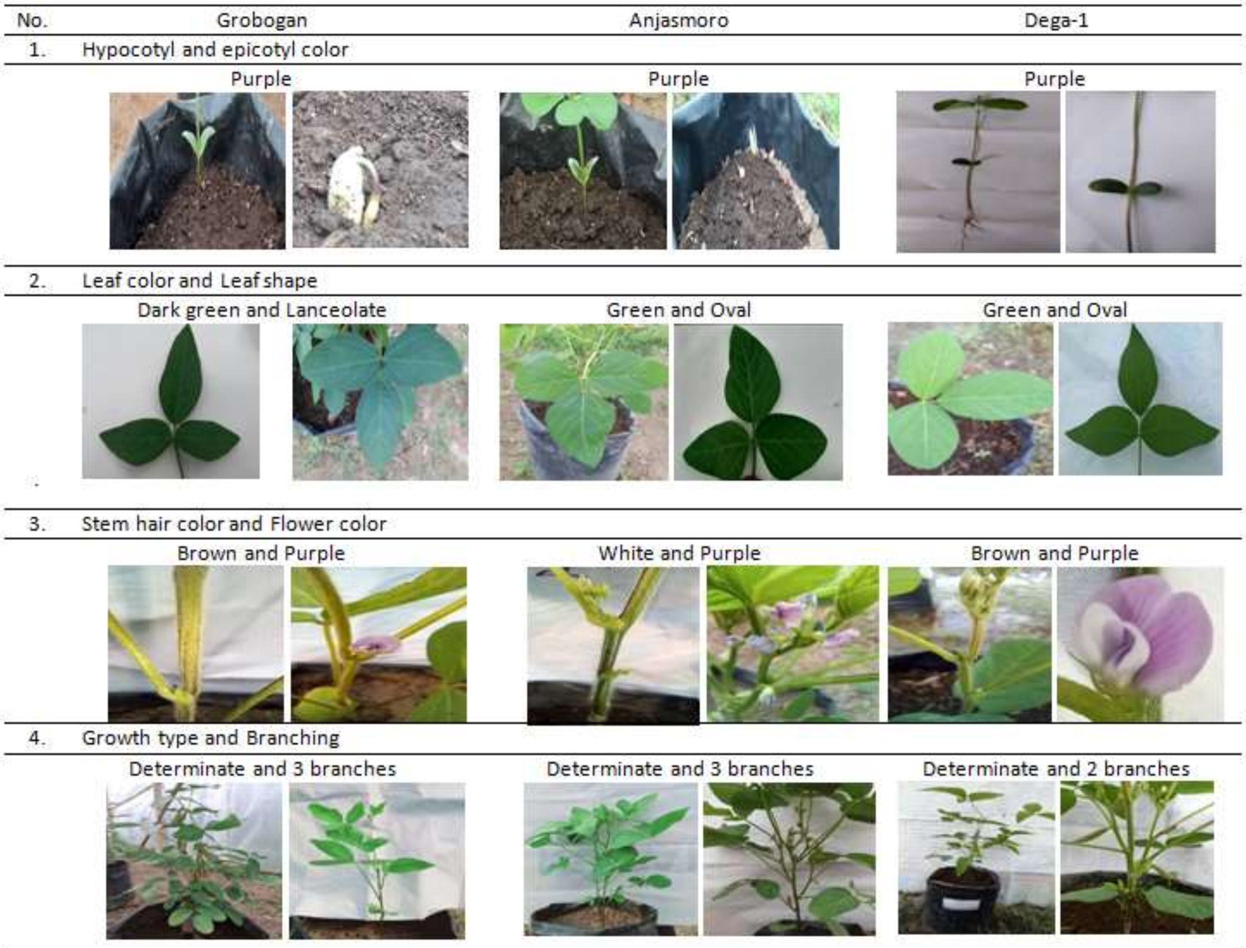


**Figure 1**. Morphological traits of soybean cultivars, i.e., Grobogan, Anjasmoro, and Dega-1.

**Table 1**. Mean values of the soybean cultivars for various morphological characters.

| Cultivars | Plant height | Primary branches | Flowering age | Number of flowers | Age of pods | Number of pods | Number of productive pods |
|---|---|---|---|---|---|---|---|
| Grobogan | 23.53 | 3.60 | 31 | 56.37 | 41.87 | 40.26 | 19.73 |
| Anjasmoro | 55.39 | 2.30 | 38 | 48.10 | 47.00 | 60.83 | 56.47 |
| Dega-1 | 56.60 | 1.80 | 28 | 14.00 | 36.00 | 37.80 | 35.40 |

the branches sprout from axillary buds. The gene expression of the low-branching soybean cultivars regulated the low axillary bud development and formation, thus, the number of plant branches finally formed were less, while the genes that regulated the development of axillary buds in multi-branching soybean cultivars were active, giving more formed branches (Bao *et al.,* 2019; Chen *et al.,* 2020).

Determining the pod number in soybean plants starts during a period around flowering and extends through the pod setting up to the beginning of the seed-filling period. Available assimilates during these post-flowering phases affect the pod's number and seeds per pod. Thus, these post-flowering phases are often considered critical periods for yield determination (Egli, 1998). In addition, Nico *et al*. (2015) stated that long days during


**Figure 2**. Soybean flowers: a) Blooming flower, b) Flower bud.

post-flowering phases increase the pod number and seeds per pod per square meter in soybean, primarily due to an increase in the production of nodes and, secondly, due to increases in pods per node, without changing the number of seeds per pod.

In the total number of pods, cultivar Anjasmoro was higher than the two others, Grobogan and Dega-1. However, the difference in the number of productive pods out of the total number of pods in Grobogan (19.73 out of 40.26) was higher than cultivar Anjasmoro (56.47 out of 60.83), revealing that the number of empty pods was higher in cultivar Grobogan than Anjasmoro. A cultivar plant can be unproductive if it has many empty pods. The genetic makeup of the genotypes, inputs used, temperature, and other environmental factors controlled the number of empty pods formed in a soybean plant during the pod formation, which boost or inhibit seed formation in pods (Nurrohman *et al*., 2017).

**Flowers**

The morphology of soybean flowers includes perfect flowers (hermaphrodite), i.e., each flower has a pistil and stamens and is very small of the self-pollinating type. Flowers located on the segments of the stem are either purple or white. Soybean flowers first bloom in the morning and then bud (Figure 2). Flowers consist of a stalk (pedicellus), additional petals (epicalyx), petals (calyx), corolla, stamens, and pistil (pistillum). Flowers bloom in the morning around 0800–0900 h. Factors that affect the age of the flower are the genetic makeup of the cultivar, temperature, and duration of irradiation. The flowering period lasts from three to five weeks. The first flower appears on the fifth book or the book above it. Flowers appear in groups of two to 35 flowers. Not all the flowers managed to form pods, about 20%–80% mostly fall (Adie and Krisnawati, 2019).

**Success in crosses**

The crosses between the soybean cultivars Grobogan and Anjasmoro have a better success rate (57.14%) (Table 2). The cross success is also due to several factors, including the receptive and anthesis period of the flowers, the cross timing, the crossing compatibility between the parental genotypes, the breeder skills, the level of plant fertility, and the planting environment. According to Lubis *et al*. (2015), the best receptive and anthesis period for soybean flowers is from 0530 to 0900 h West Indonesia time zone. A factor affecting artificial crossbreeding is the existing climatic and weather conditions. Artificial crosses should have cool air (24 °C – 28 °C), wherein the pollen will agglomerate;

**Table 2**. Percentage of single cross success in soybean genotypes.

| Cross | Number of flowers crossed | Number of successful pollinations | Success percentage |
|---|---|---|---|
| | | | ---%--- |
| Grobogan × Anjasmoro | 63 | 36 | 57.14 |

**Table 3**. Number of seeds and one-seeded pods in the soybean genotypes.

| Cross | Number of seeds in a one-seeded pod | The number of one-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| $F_1$ | 8 | 8 | 100 |

**Table 4**. Number of seeds and two-seeded pods in the soybean genotypes.

| Cross | Number of seeds in a two-seeded pod | The number of two-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| $F_1$ | 22 | 11 | 100 |

**Table 5**. Number of seeds and three-seeded pods in the soybean genotypes.

| Cross | Number of seeds in a three-seeded pod | The number of three-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| $F_1$ | 0 | 0 | 0 |

however, in hot air, the pollens and pistils quickly dry (Fatimah *et al.,* 2019).

The impediment to a successful soybean breeding is, to a large extent, by poor hybridization. The soybean flower is small, delicate, and highly sensitive to stigma injury. In general, the success rate of soybean hybridization ranges from 2%–3% to 11%–15% depending upon the crossing approach and skills, followed by the prevailing environmental conditions where the crop is grown (Shivakumar *et al*., 2016). In the presented study, the percentage of cross success was high compared with the percentage of success of crosses, in general. This higher success rate can refer to the favorable weather conditions prevailing during the crossing period and the crosses' location (Talukdar and Shivakumar, 2012).

**Seeds per pod**

The number of seeds in the crosses of Grobogan and Anjasmoro are available in Tables 3, 4, and 5. Crosses with Anjasmoro as male parents and Grobogan as female parents exhibited more success and productivity for more pods and seeds. According to Delaplane *et al*. (2013), the success of the cross shows from the formation of pods and seeds. Not all crossed flowers may produce pods and seeds because these yield components receive influences from several other factors, such as, flower fall, suboptimal crossing conditions, and pests.

The number of seeds per pod is an essential yield component that greatly influences the seed yield. The number of pods with three spores highly contributed to increasing seed weight per plant, followed by three other variables, i.e., the number of branches, the number of nodes, and the total number of fertile pods. Thus, the increasing number of pods with three seeds is closely related to enhanced seed weight per plant in soybean genotypes (Sundari and Pratiwi, 2018). The past findings of Lyimo *et al.* (2017) authenticated that the number of pods per plant strongly positively correlates with soybean yield.

The success of artificial pollination (hand pollination) in the crosses of cultivars Grobogan and Anjasmoro formed 36 pods (19 productive and 17 empty). The number of seeds (30) and the total weight of seeds (5.4 g) of crosses made from cultivars Grobogan and Anjasmoro provided the best values (Table 6). The seed weight attains influence from the N nutrient content in the soil. According to Pandiangan and Rasyad (2017), applying N fertilizer 30 days after planting (DAP) can increase seed weight because it increases assimilate translocation in the seed-filling phase.

**Cross success in three-way crosses**

Three-way crosses between $F_1$ hybrid and other soybean cultivars ([Grobogan × Anjasmoro] × Dega-1) have a better success rate (35.37%) (Table 7). The rating of such success percentage of the three parental crosses belonged in the medium success category because the results obtained were above 20% and below 60%. Based on past research of Talukdar and Shivakumar (2012), the percentage of success of artificial crosses in soybeans was 11%–15%. Lubis *et al*. (2015) stated that generally, the average percentage of success of static crosses was in the range of 20% (the smallest number) to 60% (the highest) in the soybean.

In artificial crosses (hand pollination), several factors influence the crossing process, namely, the available flowers that are not simultaneous and different from male and female parents, the timeliness of crosses, and the level of skills. Available flowers that were not simultaneous and different from male and female parents provided the number of flowers from all samples on $F_1$ descent at 369 and 159 in cultivar Dega-1. Crossings ensued in the morning and evening to have more percentage of successful crosses. No artificial crossings happen during the daytime because the pollen dries out soon, which makes it difficult to attach to the stigma.

**Seeds per pod in three-way crosses**

Based on observations, the number of seeds in the cross of $F_1$ descent (Grobogan × Anjasmoro) with Dega-1 appears in Tables 8, 9, and 10. The success rate of artificial crosses in the cross $F_1$ succession (Grobogan × Anjasmoro) × Dega-1 formed 29 pods (17 were productive and 12 were empty), with the number of seeds at 25 and the total seed weight at 5.6 g (Table 11).

The number of pods in artificial crosses had the quality and ability of pollen to fertilize the ovaries affecting it. The success in the cross and the number of pods and seeds formed are also due to the amount of pollen that falls on the stigma in soybean (Arifianto *et al*., 2015). The existence of flowers that succeed in forming pods but do not produce seeds may be due to several factors, including lack of fertilization at the time of artificial pollination and too little and weak pollen stopping to proceed for fertilization of seeds. Pest attacks and environmental factors also cause the formation of hollow pods in soybean (Lubis *et al*., 2015).

The formation of filled pods also receives photosynthesis impact. Soybean plants contain a pod consisting of one to four seeds. The fuller and larger the seeds in each pod confirm the better quality of the pods. Based on the findings of Rezyawati *et al*. (2018), the leaf area affects pod filling; the broader the leaf, the more photosynthates are translocated without any shaded leaves in soybean. Chairudin *et al*. (2015) stated that low light intensity could cause a decrease in the number of filled pods due to inhibited plant metabolism, which causes a decline in the supply of photosynthate to the seeds, decreasing the number of filled pods in soybean. The number of empty pods also affects soybean production; the more empty

**Table 6**. Number of productive pods in the soybean genotypes.

| Cross | Number of seeds in a three-seeded pod | The number of three-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| $F_1$ | 0 | 0 | 0 |

**Table 7**. Percentage of cross success from a cross of three parents in soybean.

| Cross | Number of flowers crossed | Number of successful pollinations | Success percentage |
|---|---|---|---|
| | | | ---%--- |
| $F_1$ × Dega-1 | 84 | 29 | 35.37 |

**Table 8**. The number of seeds and one-seeded pods from a cross of three parents in soybean.

| Cross | Number of seeds in a one-seeded pod | The number of one-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| F1.1 | 9 | 9 | 100 |

**Table 9**. The number of seeds and two-seeded pods from a cross of three parents in soybean.

| Cross | Number of seeds in a two-seeded pod | The number of two-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| F1.1 | 7 | 14 | 100 |

**Table 10**. Number of seeds and three-seeded pods from a cross of three parents in soybean.

| Cross | Number of seeds in a three-seeded pod | The number of three-seeded pods | Percentage of seed formation in pods |
|---|---|---|---|
| | | | ---%--- |
| F1.1 | 1 | 3 | 100 |

**Table 11**. Number of productive pods from a cross of three parents in soybean.

| Cross | Number of productive pods | Total number of seeds in Pods | Number of empty pods | Total weight of seeds (g) |
|---|---|---|---|---|
| F1.1 | 17 | 25 | 12 | 5.6 |

pods, the less soybean produced. Empty pods can occur due to limited water, nutrients, and sunlight during seed formation. Research by Chakma *et al*. (2015) signified unfilled pod formation might occur due to insufficient nutrients during the process of seed formation in the pods in soybean.

The promising results further showed that there was a significant difference in the crosses of Grobogan × Anjasmoro and $F_1$ (Grobogan × Anjasmoro) × Dega-1 for the total number of pods, number of productive pods, number of empty pods, number of seeds in pods, and the total weight of seeds. The said differences could have impacts gained from the genetic makeup of the genotypes and environmental conditions, such as, water availability and soil acidity (Pregitzer *et al*., 2013). According to Mustofa *et al*. (2020), the trait seed yield is the chief attribute that becomes the selection criteria for high-yielding soybean genotypes. Both female and male parents contribute some of the genes to their offspring; however, the influence of the female parent often extends beyond simple genetic transmission (Munganyinka *et al*., 2015).

The inheritance study of quantitative characters will help breeders to choose appropriate breeding methods and parental genotypes for the hybridization program (Singh *et al*., 2018). Significant genetic variability in the initial breeding material ensures better chances of producing desired new variants in crop plants. Accordingly, the primary objective of germplasm assessment and conservation is to collect and preserve the genetic variability in indigenous collections of various crop species, making it available to the present and future generations (Kalyan *et al*., 2017).

## CONCLUSIONS

The cross Grobogan × Anjasmoro had a reasonably high success rate (57.14%), producing numerous one-seeded and two-seeded pods and 30 seeds per plant with a total weight of 5.4 g. The success rate of the three-way cross ($F_1$ descent [Grobogan × Anjasmoro] × Dega-1) was 35.37%, which belonged in the medium success category.

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