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Inbreeding Depression and Genetic Diversity of S1 Lines Corn Plants Under Drought Stress Conditionson Dry Land

I Wayan Sudika[1*], I Wayan Sutresna[1], Dwi Ratna Anugrahwati[1], Ni Wayan Sri Suliartini[1], I Wayan Suana[2]

[1] Agroecotechnology study program, Faculty of Agriculture, Universitas Mataram, Mataram, Indonesia.
[2] Biology study program, Faculty of Mathematics and Natural Sciences, Universitas Mataram, Mataram, Indonesia.

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Corresponding Author:
I Wayan Sudika
iwsudika@unram.ac.id

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Abstract: Selfing was carried out on the F2 population of corn plants and the S1 line was produced. Selfing is carried out to form pure lines in order to create hybrids. The aim of the research was to determine the magnitude of inbreeding depression, genetic diversity and heritability of leaf angle trait, yield and harvest age of the S1 line of corn plants under drought stress conditions in dry land. The experiment was carried out from March to June 2023 in Gumantar village, North Lombok district. The design used was a randomized block design, two replications. The number of treatments was 31, namely 30 S1 lines and an F2 population. Plantings are given heavy stress. Observed variables include flowering, growth, harvest age, yield components and yield. The level of inbreeding depression is based on the F2 population and is expressed as a percent. Genetic diversity is calculated based on the results of analysis of variance at a significance level of 5 percent. The results showed that inbreeding depression in leaf angle and harvest age of the S1 lines were relatively low; while the yield is classified as high. Broad categories of genetic diversity were obtained in the yield; while the leaf angle and harvest age are relatively narrow. Broad sense heritability was relatively high in the yield; while the leaf angle is classified as medium and the harvest age is relatively low. The selection of S1 lines should be based on yield, namely lines that have the same yield as the F2 population.

Keywords: F2 populations; Genetic diversity; Heritability; Inbreeding depression; Maize crops

Introduction

The population of the superior candidate variety Sinta Unram has wide leaf angles (> 500) (S. Sudika & Parwata, 2019), so the yield is still low. This yield potential can be increased by reducing the leaf angle. Smaller leaf angles give plants the opportunity to capture light more effectively, resulting in the opportunity to increase yields (Jaya et al., 2019). Smaller leaf angles also provide the opportunity for narrower planting distances, so that the number of plants in a certain unit area is greater. Efforts to reduce the leaf angle of the prospective superior variety Sinta Unram have been carried out by Sudika et al. (2021) by crossing using the hybrid varieties NK212 and NK7328. Both varieties have a leaf angle of around 30 – 330.

Evaluation of the F1 lines resulting from crossing Sinta Unram with NK212 and NK7328 has been carried out by Sudika et al. (2021) and it is known that most of the F1 lines have smaller leaf angles compared to Sinta Unram. The F1 lines have been grown in isolation from time and place, resulting in the F2 lines. Evaluation of F2 lines has been carried out and several F2 lines have been determined for genetic recombination and a recombined F2 population has been obtained.

In the F2 population resulting from recombination, the components of genetic variation have been estimated. The results show that the dominant variance of yield characteristics, harvest age and leaf angle is

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greater than the additive variance; so the variety that will be made is a hybrid (Adeputri et al., 2023).

The formation of hybrid varieties begins with the formation of pure lines. Pure lines are formed through selfing for up to 5-6 generations. Wulan et al. (2017) said that selfing causes a decrease in vigor and a decrease in plant characteristics. Cantika et al. (2022) obtained lower plant height and cob weight without husks, cob length and number of rows compared to half-sib and full-sib. Studies of inbreeding depression on S1 inbred lines of corn plants have also been carried out by Somera et al. (2018), that there is inbreeding depression in the nature of the outcome and outcome components. The yield of shelled seeds from line S1 to line S5 decreased in corn plants. The first selfing was carried out and 30 selfing cobs were obtained. The magnitude of the inbreeding depression and the genetic diversity of these cobs are unknown. Therefore, research has been carried out which aims to determine the magnitude of inbreeding depression, genetic diversity and heritability of leaf angle, harvest age and yield of S1 lines under drought stress conditions.

Method

The research method flow chart is as follows:

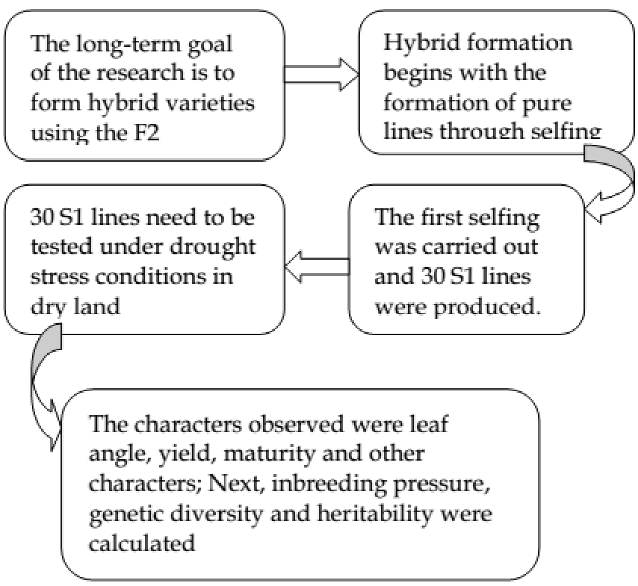

Figure 1. Research method flow

The materials used in this research were 30 seeds of the S1 line and F2 seeds, Saromyl 35 SD, Furadan 3 G, Calaris 550 SC; Meurtieur 30 EC, Proclaim 5 SG and dry land. Experimental methods were used in this research. The experiment was carried out on dry land with a pump well, namely in Amor-Amor hamlet, Gumantar village, North Lombok. The experiment was carried out from March to June 2023. The soil texture was sandy loam. The air temperature at the experimental location was 25–35^{0}C with rainfall in March, April, May and June respectively 275 mm, 242, 65 and 47 mm. A randomized block design with two replications was used in this experiment. The number of treatments was 31 consisting of 30 S1 lines (G1-G30) and F2 population (G31).

The experiment includes seed and land preparation, planting and basic fertilization, maintenance and harvest. Seed preparation was carried out by giving Saromyl 35 SD for each treatment the day before planting at a dose of 5 g/kg of seed. Land preparation is carried out by plowing and harrowing the soil once each, then leveling it. The experimental plot was divided into two blocks with the size of each block being 21.7 x 5 m. The distance between blocks is 0.50 m wide and a ditch is made 30 cm deep. Planting is done with a spacing of 20 x 70 cm, two seeds per hole. Each treatment was planted in one row 5 m long. Planting is done individually. The planting hole which has been filled with seeds, is covered with Petroganik organic fertilizer at a dose of 600 kg/ha. At the time of planting, basic fertilization is carried out with 100 kg of Urea fertilizer and 150 kg of Phonska fertilizer per hectare. Thinning is done at the age of 12 days, leaving one plant that grows better. Additional fertilization is carried out individually between plants in rows at the age of 28 days with 100 kg of Urea and 150 kg of Phonska. Irrigation is carried out the day before planting, 10 days, 17, 24, 31 and 38 days after planting by dileb method. The water source comes from a pump well.

Prevention of downy mildew is done by seed treatment using Saromyl. Control of male flower borer pests is done by spraying Meurteiur insecticide at the age of 21 days and 35 days after planting. Control of stem borers and earworms with Proclaim 5 SG insecticide. Harvesting is carried out after 85% of the plants in each row show the harvest criteria, namely the husks are dry and if the seeds are pressed with a fingernail there will be no trace. Observed variables included flowering variables (50 % anthesis, 50 % silking, anthesisi silking interval=ASI), growth (leaf angle, plant height, number of leaves, leaf area, stem diameter), harvest age and variables Yield components (cob length, cob diameter, harvested dry cob weight, weight of 1,000 kernels) and yield (dry seed weight per plot). The experimental data were analyzed using analysis of variance at a significance level of 5 percent. The results of the analysis of variance are used to calculate the genetic diversity coefficient and broad-sense heritability values.

Inbreeding depression is calculated by the equation 1.

$$DS\ (\%) = \{(F2 - S1)/F2\} * 100\ \% \quad (1)$$

With DS, it is inbreeding depression; F2, the average of the F2 population and S1, is the average of the S1 lines. Inbreeding depression categories, as proposed by Wulan et al. (Wulan et al., 2017), namely high inbreeding depression, ≥ 50.0 %; currently, 20.0 - <50.0 % and low inbreeding depression, <20.0 %.

The broad sense heritability value (H2) is calculated based on the formula proposed by Ujianto et al. (2020), as equation 2, 3, and 4.

$$H^2 = \sigma^2_g/\sigma^2_p \quad (2)$$

$$\text{The size } \sigma^2_g = (KTp - KT_G)/r \quad (3)$$

$$\sigma^2_p = \sigma^2_g + KT_G \quad (4)$$

With KT_P, is the mean squared treatment and KT_G is the mean squared error. The assessment of heritability values in a broad sense follows the categories according to Stanfield (1991), in Sudika et al. (2021), namely low heritability, < 20 %;medium, 20 – 50 % and high heritability when> 50 %.

The genetic diversity coefficient (KKG) value is calculated using the formula Ujianto et al. (2020):

$$KKG\ (\%) = (\sigma_g / \mu) \times 100\ \% \quad (5)$$

where σ_g is the genetic standard deviation and μ is the general mean. The genetic diversity criteria are based on the KKG value as stated by Bartaula et al. (2019) and Lamichhane et al. (2021), namely wide genetic diversity if the KKG value is > 20.0%; moderate, 10.0-20.0 % and narrow genetic diversity if the KKG value is <10 %.

The correlation coefficient (r) value between the observed traits, each with leaf angle, harvest age and yield, was obtained using the formula as proposed by Paiman (2019), equation 6.

$$r_{xy} = \frac{n\,\Sigma XiYi - \Sigma Xi\Sigma Yi}{\sqrt{n\Sigma Xi2 - (\Sigma Xi)^2}\sqrt{n\,\Sigma Yi2 - (\Sigma Yi)^2}} \quad (6)$$

Where: rxy is the correlation coefficient between traits X and Y. The correlation criteria based on the coefficient value follows as stated by Kurnia et al. (2021), viz 0 ≤ r < 0.20,very weak; 0.20 ≤ r < 0.40, weak; 0.4 ≤ r <0.60, currently; 0.60 ≤ r < 0.80,strong and 0.80 ≤ r < 1.0 is very strong.

Result and Discussion

Inbreeding in corn plants causes a decrease in vigor and fertility in the off spring and this event is called inbreeding depression (Charlesworth & Willis, 2009). Testing the S1 line under drought stress conditions is very necessary to form drought resistant hybrid varieties. Based on the analysis of the results, it can be seen The percentage of inbreeding depression, coefficient of genetic diversity and broad sense heritability values for all observed traits are presented respectively in Tables 1, 2 and 3.

Table 1. Inbreeding Depression in All Traits Observed Under Stress Conditions Drought on Dry Land

Observed properties	Inbreeding depression (%)	Category
50 % Anthesis	-1.08	Low
50 % silking	-1.87	Low
ASI	-16.13	Low
Leaf angle	0.14	Low
Plant height	21.04	Currently
Number of leaves	12.03	Low
Leaf area	26.81	Currently
Stem diameter	10.66	Low
Harvest age (maturity)	3.84	Low
Cob length	22.49	Currently
Cob diameter	17.75	Low
Harvested dry cob weight per plant	34.06	Currently
Weight of 1,000 seeds	21.77	Currently
Yield (dry seed weight per plot)	55.14	Tall

In Table 1, it can be seen that the inbreeding depression has a negative value, obtained at the 50 % anthesis, 50 % silking and ASI. Negative value indicates that the S1 lines flower more slowly than the population without selfing (population F2). The ASI of the S1 line is greater than that of the F2 population. The same thing was obtained by Rahmawati et al. (2016), that the average 50 % silking in the selfing treatment was slower than that oft he open pollinated variety. Widanni et al. (2019) also found that the 50 % anthesis, 50 % silking and the age at harvest were slower in these all lines. Yield characteristics (dry seed weight per plot) had inbreeding depression in the high category, namely 55.14%. This means that the yield of the S1 line is lower than the yield of the F2 population. The lower yield in the S1 lines was caused by heterozygous loci changing in to homozygous loci due to selfing.

This is in accordance with the opinion of Wulan et al. (2017), that selfing causes a decrease in vigor and a decrease in plant traits. Cantika et al. (2022) obtained lower plant height and cob weight without husks, cob length and number of rows compared to half-sib and full-sib. Other traits have inbreeding depression categories in low and moderate. The size of the inbreeding depression really depends on the number of heterozygous loci that change into homozygous loci.

Heterozygous loci controlling yield traits mostly change to homozygous loci; while what changed the least wasthe angle of the leaves. Drought stress also affects the appearance of corn plant traits. The yield of S1 lines was drastically reduced under drought stress conditions. This was proven by testing 30 S1 lines which under normal conditions experienced smaller inbreeding depression, namely 30.10 percent (I. W. Sudika et al., 2023).

It is very important to know the genetic diversity of S1 lines as a consideration in selecting lines. The genetic diversity coefficient value sand their categories are presented in Table 2.

The criteria for genetic diversity according to the KKG value follow those stated by Bartaula et al. (2019) and Lamichhane et al. (2021), namely narrow genetic diversity < 10%; moderate, 10-20% and wide genetic diversity if the KKG value is > 20%. Based on this, the yield have wide genetic diversity; while ASI and harvested dry cob weight the genetic diversity was classified as moderate. The same thing was obtained by several researchers, that the results had wide genetic diversity, namely Adhikari et al. (2018) obtained a KKG value of 21.96% for 13 corn genotypes; Rai et al. (2021) amounting to 31.53%; Kandel et al. (2018) earned 32.84 % of the two-season average.

Table 2. Genetic Diversity Coefficient (KKG) Values for the S1 Line for Each Trait Observed Under Drought Stress Conditions

Observed properties	KKG (%)	Category
50% anthesis	1.50	Narrow
50 % silking	1.89	Narrow
ASI	15.85	Moderate
Leaf angle	8.95	Narrow
Plant height	3.20	Narrow
Number of leaves	3.66	Narrow
Leaf area	8.90	Narrow
Stem diameter	4.81	Narrow
Harvest age	1.49	Narrow
Cob length	3.26	Narrow
Cob diameter	6.20	Narrow
Harvested dry cob weight	12.56	Moderate
Weight of 1,000 seeds	5.01	Narrow
Yield (dry seed weight per plot)	24.28	Wide

Other traits, namely 50 % anthesis, 50 % silking, leaf angle, plant height, number of leaves, leaf area, stem diameter, harvest age, cob diameter, cob length and weight of 1,000 seeds have narrow genetic diversity. The same thing for plant height, 50 % silking, harvest age, cob diameter and cob length, was obtained by Azrai et al. (2016) Plant height has a narrow genetic diversity also obtained by Al-Amin et al. (2019). The genetic diversity for yield traits is wide, showing that the S1 lines have high genetic variation. This can be used as a basis for selecting S1 lines that will carry out selfing in the second generation.

Table 3. Genetic Variance (σ^2_g), Phenotypic Variance (σ^2_p) and Broad Sense Heritability Value (H^2) of S1 Lines for Each Observed Trait

Observed properties	(σ^2_g)	(σ^2_p)	H^2 (%)	Category
50 % anthesis	0.40	2.74	14.7	Low
50 % silking	0.72	3.6	19.99	Low
ASI	0.13	1.38	9.80	Low
Leaf angle	7.42	25.74	28.84	Medium
Plant height	14.14	119.85	11.80	Low
Number of leaves	0.14	0.38	35.89	Medium
Leaf area	440.43	1460.27	30.16	Medium
Stem diameter	0.01	0.05	10.61	Low
Harvest age	1.09	88.77	1.23	Low
Cob length	0.08	0.62	13.24	Low
Cob diameter	0.04	0.12	30.95	Medium
Harvested dry cob weight	62.45	189.21	33.01	Medium
Weight of 1,000 seeds	42.59	544.31	8.01	Low
Yield (dry seed weight per plot)	55.03	80.03	68.76	Tall

Estimating heritability values is used to determine the role of genetic factors in the visible phenotype. Broad sense heritability indicates that the total genetic variance is used in the calculation (Syukur & Yunianti, 2012). According to Yali (2022), heritability is the proportion of genetic variation to phenotypic variation. In Table 3, broad sense heritability of the low criteria was obtained for 50 % anthesis, 50 % silking, ASI, plant height, stem diameter, harvest age, cob length and weight of 1,000 seeds. This means that genetic factors play a small role in the appearance of this character. These traits are difficult to pass on to the next generation (Prabowo et al., 2016). Leaf angle, number of leaves per plant, leaf area, cob diameter and harvested dry ear weight per plant have medium heritability criteria.

Medium heritability means that environmental factors and genetic factors contribute equally to the character (Rosliana et al., 2018). Sudika et al. (2021), obtained the same thing for harvest dry cob weight per plant with medium broad sense heritability criteria. The yield trait (dry seed weight per plot) has high criterion heritability. According to Fehr (1987), a high heritability value for a character indicates that the character's appearance is more determined by genetic factors. Characters with high heritability will appear in their offspring in the early generations. Sudika et al. (2023), also obtained high category heritability for the yield of selected composite corn, namely 64.53% for the Pererenan location and 51.18% for the Amor-Amor location.

The relationship between traits with leaf angle, harvest age and yield are important when considering selecting the S1 lines. The correlation coefficient values and criteria are presented in Table 4. In Table 4 it can be seen that all the observed traits have no correlation with leaf angle under drought stress conditions, with very weak criteria.

There is mostly no correlation between the observed traits and harvest age, with very weak criteria. Two characteristics have a correlation with harvest age, namely cob diameter with strong criteria and harvested dry ear weight with weak criteria. The implication is that strain selection is carried out directly by looking at the size of the leaf angle and the age of harvest. The selected line is a line with a leaf angle of < 35^0 and has a harvest age of < 80 days. Yield was positively correlated with plant height, number of leaves, leaf area, ear length, ear diameter, harvested dry ear weight per plant and weight of 1.000 seeds. The criteria for a strong correlation with the results were obtained in the characteristics of leaf area, ear length and harvested dry ear weight per plant. This means that the wider the leaves, the higher the yield.

Table 4. Correlation Coefficient Values between Observed Traits with Leaf Angle, Harvest Age and Yield of S1 Line Under Drought Stress Conditions

Observed properties	Correlation coefficient					
	With leaf angle	Criteria	With harvest age	Criteria	With result	Criteria
50 % anthesis	0.00	Very weak	-0.14ns	Very weak	-0.16ns	Very weak
50 % silking	0.08	Very weak	0.16ns	Very weak	-0.17ns	Very weak
ASI	0.01ns	Very weak	-0.09ns	Very weak	-0.04ns	Very weak
Leaf angle	1.00		0.21ns	Weak	-0.07ns	Very weak
Plant height	-0.15ns	Very weak	-0.07ns	Very weak	0.56s	Currently
Number of leaves	-0.03ns	Very weak	0.11ns	Very weak	0.30s	Weak
Leaf area	0.04ns	Very weak	0.07ns	Very weak	0.80s	Strong
Stem diameter	0.01ns	Very weak	-0.02ns	Very weak	0.12ns	Very weak
Harvest age	0.21ns	Very weak	1.00		0.03ns	Very weak
Cob length	0.035ns	Very weak	0.12ns	Very weak	0.74s	Strong
Cob diameter	0.12ns	Very weak	0.81s	Strong	0.47s	Currently
Harvested dry cob weight	0.01ns	Very weak	0.25s	Weak	0.80s	Strong
Weight of 1,000 seeds	0.03ns	Very weak	-0.02ns	Very weak	0.43s	Currently
Results	-0.03ns	Very weak	0.03ns	Very weak	1.00	

Note: Table r value 5% = 0.25; s, there is correlation and ns, there is no correlation.

Likewise, the longer the cobs are and the higher the weight of harvested dry cobs per plant, the higher the yield. If a line has a leaf angle of < 350 and a harvest age of <80 days, but the yield is low, then the ear length and harvested dry ear weight can be used to determine the selected S1 lines. The same thing was obtained by Hikmah et al. (2023), who carried out tests under normal conditions on dry land. A real positive correlation with the yield was obtained in the characteristics of plant height (0.556), leaf area (0.796), cob length (0.743), cob diameter (0.470), harvest dry ear weight per plant (0.802) and weight of 1,000 seeds (0.429) Kamal et al. (2020), obtained the same thing for the correlation between cob length and yield, namely a positive correlation of 0.63. Hadi et al. (2021) obtained a correlation coefficient of yield with cob length of 0.681.

Sudika et al. (2023) obtained the same thing, that the weight of harvested dry cobs was positively correlated with yield, but the magnitude was different by 0.47 with medium criteria. Crevelari et al. (2018) obtained a correlation coefficient value for the weight of harvested dry cobs with yield of 0.71 with strong criteria. Yield with 50 % anthesis, 50 % silking and ASI were not correlated, respectively at 0.160, 0.170 and 0.040. Chandana et al. (2018) obtained the same thing, that there was no correlation between the yield at 50% anthesis, 50% silking and ASI, respectively 0.041, 0.047, and 0.029.

Conclusion

Inbreeding depression in leaf angle and harvest age of the S1 lines were low; while yield were classified as high under drought stress conditions. Broad categories of genetic diversity were obtained in the yield; while the leaf angle and harvest age are relatively narrow. Broad sense heritability was relatively high in the yield; while the leaf angle is classified as medium and the harvest age relatively low. The S1 lines, which had the same yield as the F2 population, was designated as the selected line, namely 28 lines. These lines were selfed to obtain the S2 line.

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Author Contributions

Conceptualization; I. W. S, I. W. S, D. R. A, N. W. S.; methodology.; I. W. S.; validation;I. W. S.; formal analysis; D. R. A.; investigation.;N. W. S.; resources; I. W. S; data curation: I. W. S.; writing—original draft preparation.D. R. A.; writing—review and editing: N. W. S.; visualization: I. W. S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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