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Tolerance Level of S4 Corn Lines to Drought Stress in Dry Land

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Abstract: Selfing is the initial step to form hybrid varieties. Selfing has been carried out for four generations and 29 S4 lines have been obtained. All lines have been tested for their tolerance level to drought stress in dry land. The purpose of the study was to determine the tolerance level of S4 lines of corn plants to drought stress in dry land. Experimental methods with field trials were used for the test. And the correlation between characters and ISC values. The design used was a randomized block design at each stress level. Determination of the tolerance level used the observation parameter of the results (dry seed weight per plant) under mild stress conditions and normal conditions. The tolerance level to drought stress was measured from the Stress Sensitivity Index (ISC) value. The results showed that three S4 lines were tolerant to drought stress, namely lines S4.7, S4.15 and S4.24. Nine S4 lines have moderately tolerant category, namely lines S4.1, S4.3, S4.8, S4.12, S4.13, S4.14, S4.21, S4.26 and S4.29; the rest are classified as sensitive. The yield have a high negative correlation with the ISC value; the weight of dry cobs harvested per plant has a moderate negative correlation. The three tolerant lines can be used for the formation of S5 lines.

Keywords: Drought stress; Maize plants; S4 line; Tolerance

Introduction

Increasing corn production continues to be pursued through intensification and extensification. Both efforts require the development of hybrid varieties (Chakrabarty et al., 2023). The use of hybrid varieties is the main key to increasing corn productivity in Indonesia (Bahtiar et al., 2023; Syahruddin et al., 2020). This is done considering that hybrid corn has a higher yield potential than open-pollinated varieties. Increasing production through intensification and extensification can be done on dry land (Baudron et al., 2021; Marinus et al., 2023). Therefore, efforts are needed to form superior hybrid varieties that are adaptive to dry land. The formation of super early maturing or early maturing hybrid varieties needs to be done to increase corn production in Indonesia, especially on dry land (Anshori et al., 2024). The formation of a corn population to obtain superior super early maturing hybrid varieties has been carried out by Sudika & Anugrahwati (2021). This activity has produced an F2 population and its genetic variation components have been suspected. The results of the study showed that the dominant variation was higher than the additive variation for leaf angle and yield properties; however, for harvest age; both varieties are the same. Based on this, it is recommended to form hybrid varieties (Duruflé et al., 2023).

The formation of hybrid varieties begins with the formation of inbred lines (Labroo et al., 2021). The formation of inbred lines is carried out by selfing until homozygous lines are obtained (Arrones et al., 2020; Yan et al., 2017; Zhang et al., 2022). The first, second and third generation selfings have been carried out and 15 S3 lines have been produced. Testing of the S1 line has also been carried out and it is known that the results have moderate cross depression, namely 30.10%. The leaf angle and harvest age of the deep cross depression are low, namely 8.18% and -0.02% respectively (Lopes et al., 2022). Testing of the S1 line has been carried out by (Chaikam et al., 2019), to obtain lines that are tolerant to drought stress. The results of the study showed that as many as 14 S1 lines were sensitive, 13 moderate and one

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tolerant line, namely G27 to severe drought stress. (Adebayo & Menkir, 2014; Adee et al., 2016; Walne et al., 2024), obtained that of the six hybrid corn tested under drought stress, two tolerant hybrid varieties, namely SC647 and KSC704, were based on the sensitivity index.

Testing of 20 corn genotypes under drought stress was conducted by Chiango et al., 2021; Shojaei et al., 2022). The results of the study showed that ASI was longer, as a result of the slow emergence of cob hairs. The fourth generation selfing activity was carried out to form S4 lines using 15 S3 lines. The number of S4 lines obtained was 29 lines. The tolerance of these lines was tested on dry land to obtain pure lines that were tolerant to drought stress. The purpose of the study was to determine the tolerance level of the fourth generation selfing lines (S4) of corn plants on dry land.

Method

The materials used in this experiment were 29 S4 corn seeds, raffia rope, Urea fertilizer, Phonska fertilizer, Petroganik, Saromyl 35 SD, Furadan 3G, Meurtieur 30 EC, Calaris 550 SC and plastic bags. The method used in this study was an experimental method with field experiments, namely on dry land that had a pump well. The experiment was conducted in the Amor-Amor hamlet, Gumantar village, North Lombok district. The experiment began in early June to the end of August 2024. The design used in each condition (normal and mild drought stress) was a Randomized Block Design (RAK). The number of S4 lines tested was 29; each as a treatment. Each treatment was repeated twice, so that 58 experimental units were obtained for each stress condition. Given the number of stress conditions as many as two, the number of experimental units was 156. Stages of research implementation, as shown in Figure 1.

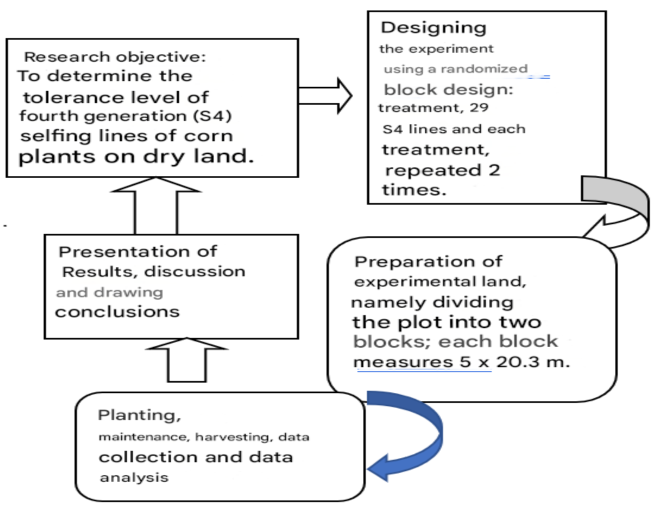

Figure 1. Stages of research implementation

The implementation of the experiment includes seed and land preparation, planting, fertilization, irrigation, pest control, harvesting and post-harvest. Seed preparation is carried out by treating the seeds of each line using Saromyl 35 SD with a dose of 5 g/kg of seed. Soil processing is carried out by plowing and harrowing once each, then the soil is leveled with a hoe. The plot for normal condition treatment is divided into two blocks; each block measures 5 x 20.3 m and the distance between blocks is 1 m. Planting and thinning. Planting by digging using a planting distance of 70 x 20 cm, two seeds per hole. The planting hole is covered with Petroganik organic fertilizer with a dose of 600 kg/ha. Each treatment is planted in one row; each row contains 25 plants. At the age of 12 days, thinning is carried out and one plant with better growth is left. Fertilization is carried out twice, namely when planting and 28 days after planting. The dosage for each fertilization is 100 kg of Urea and 150 kg of Phonska. Irrigation in normal plots is done one day before planting, at the ages of 10 days, 17, 24, 35, 42, 49, 56, 63 and 70 days. Irrigation in mild drought stress plots is done one day before planting, at the ages of 10 days, 17, 24, 30, 60, 67 and 74 days after planting. Irrigation is done in a more, using a pump well as a water source.

Pest control is done using Furadan 3 G; which is given to the planting hole. At the ages of 35 days and 56 days, spraying is carried out to control caterpillar pests using Meurtieur 30 EC with a dose of 3 cc / l of water. Control of downy mildew disease with seed treatment using Saromyl 35 SD. Weeds were controlled by spraying Calaris 550 SC 14 days after planting and by hilling up at 28 days after planting. Observed variables included flowering and harvest age, growth and yield component variables and yield. Flowering variables include panicle emergence age, cob hair emergence age and the difference between cob hair emergence and panicle emergence (ASI) and harvest age. Observed growth variables consisted of plant height, number of leaves per plant, leaf angle, stem diameter and leaf area. Observations for yield component variables, namely cob length, cob diameter, dry harvest cob weight and weight of 1.000 seeds. Results were measured from the weight of dry kernels per plant with a water content of 14 percent. Data analysis was carried out by calculating the Stress Sensitivity Index (SSI) with the formula as stated by (Nandhini et al., 2022), with the following Formula:

$$ISC = ((1 - (Yp/Y) / (1 - (Xp/X)) \quad (1)$$

with; Yp, is the average yield of a line in a stressed environment; Y, the average yield of a line in a normal environment; Xp, the average yield of all lines in a stressed environment and X, is the average yield of all lines in a normal environment. The category of resistance level according to the value, namely tolerant: ISC ≤ 0.50; moderately tolerant: ISC > 0.50 – 1.00 and sensitive ISC value: > 1.00. In order to determine the closeness of the relationship between the characters observed in drought stress conditions with the ISC value, the Pearson correlation coefficient calculation was carried out. This is needed to support the study of drought stress resistance of the S4 line. Testing the correlation coefficient value is done by comparing the correlation coefficient value with r0.05.

The number of data used to calculate the correlation coefficient value is 29, so that n for the r table is 27. Based on this, r0.05 (27) is 0.37. Next, correlation categories were created, namely low, medium and high, following the method used by Taber (2018) using r0.05. The lowest limit that was significantly different was used as the correlation criterion, namely 0.38. The range of low category values: 0.38-0.59; medium: 0.60 - 0.81 and high correlation >0.81. The S4 line that will be used to form the S5 line, needs to be selected based on its tolerance level to drought stress, leaf angle, harvest age. The requirements for the selected S4 line, namely having a leaf angle <350, super early harvest age (≤ 80 days) with a tolerance level category classified as tolerant.

Results and Discussion

The results of the calculation of the stress sensitivity index (ISC) and tolerance level categories are presented in Table 1; while Table 2 presents the results of the average t-test of each character under drought stress and normal conditions and the percentage difference compared to normal conditions. The closeness of the relationship between characters with ISC values and results is presented in Table 3. The selection of the S4 line to form the S5 line is not only based on the tolerance category, but also based on the average leaf angle and harvest age; both characters are presented in Table 4.

Tolerance level of the S4 line to drought stress

One of the characteristics of superior hybrid corn varieties for dry land is tolerance to drought stress. Therefore, candidate pure lines should also have this tolerant trait. Testing the level of tolerance to drought stress has been carried out on dry land using mild drought stress. Mild drought stress is carried out by not providing water from 12 days before flowering to 12 days after flowering (Chen et al., 2023; Ishimaru et al., 2022; Liang et al., 2019). In this experiment, mild drought stress, the last irrigation was at 30 days old and re-irrigated at 60 days old with an interval of 5 days. Table 1 shows that there are three lines that have a tolerance level with a tolerant category, namely lines S4.7, S4.15 and S4.24 with ISC values ranging from 0.08-0.44. The moderately tolerant category, obtained 9 lines, namely lines S4.1, S4.3, S4.8, S4.12, S4.13, S4.14, S4.21, S4.26 and S4.29 with ISC values ranging from 0.61-1.00; A total of 17 S4 lines have a sensitive category, namely S4.2, S4.4, S4.5, S4.6, S4.9, S4.10, S4.11, S4.16, S4.17, S4.18, S4.19, S4.20, S4.22, S4.23, S4.25, S4.27 and S4.28. with an ISC value >1.00.

The tolerance level category is based on the stress sensitivity index (ISC) value. The ISC value is calculated from the results under drought stress conditions and normal conditions of each line and the average of all S4 lines. The greater the difference in the yield of a line under the two stress conditions, the greater the ISC value; meaning that the line is more sensitive to drought stress. (Aulia Adeputri et al., 2024; Asargew et al., 2024), tested 30 S1 lines under severe drought stress conditions in dry land and obtained only one tolerant line and 13 moderately tolerant lines while 16 S1 lines were classified as sensitive.

Table 1. ISC values and tolerance levels to drought stress of S4 corn lines

Tested strains	ISC Value	Tolerance level
S4.1	0.65	Moderately tolerant
S4.2	1.40	Sensitive
S4.3	0.61	Moderately tolerant
S4.4	1.44	Sensitive
S4.5	1.24	Sensitive
S4.6	1.23	Sensitive
S4.7	0.08	Tolerant
S4.8	0.67	Moderately tolerant
S4.9	1.03	Sensitive
S4.10	1.24	Sensitive
S4.11	1.04	Sensitive
S4.12	0.68	Moderately tolerant
S4.13	1.00	Moderately tolerant
S4.14	0.67	Moderately tolerant
S4.15	0.44	Tolerant
S4.16	1.24	Sensitive
S4.17	1.37	Sensitive
S4.18	1.15	Tolerant
S4.19	1.40	Sensitive
S4.20	1.09	Sensitive
S4.21	0.78	Moderately tolerant
S4.22	1.10	Sensitive
S4.23	1.18	Sensitive
S4.24	0.24	Tolerant
S4.25	1.54	Sensitive
S4.26	0.63	Moderately tolerant
S4.27	1.51	Sensitive
S4.28	1.14	Sensitive
S4.29	0.93	Moderately tolerant

The size of the ISC value is related to several characters as presented in Table 2. The cob diameter has a low negative correlation with the ISC; while the cob length and dry harvest cob weight have a moderate negative correlation and the yield has a high negative correlation with the ISC value. The correlation coefficient values of cob diameter, cob length, cob weight and yield with the ISC value are respectively -0.58; -0.72; -0.78 and -0.84. This means that the higher the yield, the lower the ISC value; meaning that the line is more tolerant to drought stress. Longer and heavier cobs have quite an effect on the ISC value, namely the smaller it is. The size of the cob diameter has little effect on the ISC value. Other characters are not correlated with the ISC value; meaning that the size of the character value does not affect the ISC value (Ren et al., 2022), found that the cob length has a moderate negative correlation with the ISC; while the dry harvest cob weight and yield have a strong negative correlation with the ISC in the S1 line on dry land.

Table 2. Correlation coefficient values between quantitative characters of S4 corn line and ISC values

Quantitative characters observed	Correlation Coefficient Value with ISC	Correlation coefficient value with results
Age of anthesis	-0.06 ns	-0.08 ns
Age of silking	-0.18 ns	-0.07 ns
ASI (difference between age of silking and age of anthesis)	-0.19 ns	0.04 ns
Harvest age	0.07 ns	-0.06 ns
Plant height	-0.29 ns	0.43*
Number of leaves per plant	0.19 ns	-0.25 ns
Leaf angle	-0.04 ns	0.08 ns
Stem diameter	-0.24 ns	0.12 ns
Leaf area	-0.33 ns	0.50 *
Cob length	-0.72 **	0.82***
Cob diameter	-0.58 *	0.76 **
Dry harvest cob weight per plant	-0.78 **	0.95 ***
Weight of 1,000 grains	-0.12 ns	0.19 ns
Yield (dry kernel weight per plant)	-0.84***	1.00

Description: r0.05 value (27) = 0.37; ns, uncorrelated; *, low correlation and **, medium correlation and ***, high correlation.

The yield (dry kernel weight per plant) are highly positively correlated with cob length and dry cob weight at harvest; while cob diameter is positively correlated in the medium category and plant height and leaf area are positively correlated in the low category with yield. This shows that the contribution of plant height and leaf area is small to the yield; namely the S4 line of corn plants; which has taller plants and wider leaves, has little contribution to increasing yields. Chang et al. (2021); Li et al. (2021), obtained that the yield are positively correlated in the medium category with plant height, leaf area, cob length and cob diameter; while the dry cob weight at harvest per plant is strongly positively correlated. Research on F2 lines of corn plants has been conducted by Sudika et al. (2022a); Sudika et al. (2022b), it was found that plant height had a weak positive correlation with yield, leaf area, cob length and cob diameter had a moderate positive correlation and dry harvest cob weight had a strong positive correlation with yield. Other characters, such as plant height, leaf angle and stem diameter were not correlated with ISC values (Badaruddin et al., 2017) found that plant height, leaf angle and stem diameter were not correlated with yield under stress and normal conditions.

Effect of drought stress on flowering and harvest age, growth, yield components and yield in S4 lines

Differences in tolerance levels of each S4 line occurred because the difference in yield (dry seed weight per plant) obtained under drought stress conditions and normal conditions was different for each line. Differences in several other characters also contributed to the level of tolerance of the lines to drought stress. The results of the average t-test of each character under drought stress and normal conditions and the percentage difference compared to normal conditions are presented in Table 3.

Table 3 shows that there is a difference between the average characters in drought stress conditions and normal conditions based on the t0.05 test. The different characters are age of anthesis, age of silking, ASI, harvest age, cob length, cob diameter, dry cob harvest weight per plant, weight of 1.000 seeds and yield. All growth variable characters are the same between drought stress conditions and normal conditions. The percentage difference in the average varies with a range of values from 0.00% in leaf angle and stem diameter to 206.35 percent in ASI. Flowering variables in the S4 corn line that were observed included age of anthesis, age of silking and ASI. These three characters are influenced by drought stress (Table 3).

The S4 line tested under drought stress conditions produced anthesis and silking earlier than under normal conditions. The same thing was obtained by (Ali-Dinar et al., 2021), that male flowers emerged earlier than under normal conditions. ASI under drought stress conditions was greater than ASI under normal conditions, respectively 3.28 and 1.07 (Table 3). (Utami et al., 2023) also obtained the same thing, that the G7 line had an ASI of 1.67 under normal conditions and 3.00 under moderate drought stress conditions. Nisfiyah et al. (2024), obtained that the age of male flowers and the age of female flowers were influenced by the combination treatment of seed coating and the level of drought stress in superior Madura corn plants (MDR-3). The growth variables measured included plant height, number of leaves per plant, leaf angle, stem diameter and leaf area. All of these characters were the same between drought stress conditions and normal conditions. According to Jahan et al. (2023); Yan et al., (2024), that drought stress affects the rate of photosynthesis and aging of plant organs. The presence of mild drought stress in this study did not cause a difference in the rate of photosynthesis, so that the average vegetative growth of the two stress conditions was the same. Muazam et al. (2023) found that the stem diameter and number of leaves per plant were the same between drought stress conditions and normal conditions in local corn from Southeast Sulawesi.

Table 3. The average of all characters of the S4 line under drought stress and normal conditions on dry land and the results of the t0.05 test and the percentage difference for each character

Observed characters	Stress conditions	Normal condition	Test Results $t_{0.05}$	Percentage difference (%) *
Age of anthesis (days)	41.26	44.53	s	7.34
Age of silking (days)	44.53	45.60	s	2.35
ASI (days)	3.28	1.07	s	206.54
Harvest age (days)	74.50	76.07	s	2.06
Plant height (cm)	180.12	178.90	ns	0.68
Number of leaves per plant (strand)	12.59	12.63	ns	0.32
Leaf angle (0)	28.26	28.26	ns	0.00
Stem diameter (cm)	1.57	1.57	ns	0.00
Leaf area (cm2)	374.34	373.92	ns	0.11
Cob length (cm)	9.85	12.87	s	23.47
Cob diameter (cm)	3.85	4.55	s	15.38
Dry harvest cob weight per plant (g)	79.34	128.18	s	38.10
Weight of 1,000 grains (g)	225.98	246.14	s	8.19
Yield (dry kernel weight per plant (g)	42.35	69.60	s	39.15

Note: *, is the percentage difference in stress and normal conditions compared to normal conditions.

The observed yield components included cob length, cob diameter, dry harvest cob weight, and weight of 1.000 seeds. These four characters have higher values under normal conditions than under drought stress conditions. Nisfiyah et al. (2024) found that cob length, cob diameter, and yield were influenced by a combination of seed coating and drought stress. The yield of the S4 corn line under drought stress conditions was lower than under normal conditions with a yield decrease of 39.15 percent (Sudika et al., 2023) obtained the same thing, that the length of the cob, the diameter of the cob, the weight of 1.00 seeds and the yield differed between mild stress conditions and normal conditions with a decrease in yield of 32.56–39.86% in tolerant genotypes and 50.89–88.33% in sensitive genotypes. In drought stress conditions, there is a decrease in the rate of photosynthesis, so that less photosynthate is produced. In addition, there is also an obstacle to the transport of feedback to the sink. This causes the cob to be shorter and smaller, the seed size is also smaller, so the yield is lower.

Selection of S4 lines for the formation of S5 lines

The selected S4 lines, in addition to considering the level of tolerance to drought stress, are also based on the size of the leaf angle and the harvest age of the line. The requirements for selected lines are that they have a tolerant category, a leaf angle <350 and a harvest age of around 70-80 days. The average leaf angle and harvest age of each S4 line are presented in Table 4.

Table 4. Average leaf angle (0) and harvest age (days) of each S4 line of corn plants in dry land

Tested strains	Leaf angle (^{0})	Harvest age (days)
S4.1	28.50	73.50
S4.2	28.00	73.00
S4.3	31.75	73.00
S4.4	28.00	74.50
S4.5	28.25	76.50
S4.6	26.50	74.00

Tested strains	Leaf angle (^{0})	Harvest age (days)
S4.7	28.75	74.00
S4.8	27.50	73.50
S4.9	28.10	74.00
S4.10	25.00	74.00
S4.11	28.25	74.50
S4.12	26.75	74.00
S4.13	27.00	74.50
S4.14	29.50	75.00
S4.15	26.50	75.50
S4.16	28.50	75.00
S4.17	29.50	74.00
S4.18	27.25	76.00
S4.19	28.25	72.50
S4.20	29.75	75.50
S4.21	28.25	74.00
S4.22	27.00	76.50
S4.23	31.75	73.50
S4.24	28.75	75.00
S4.25	28.50	75.50
S4.26	28.75	76.00
S4.27	30.00	76.00
S4.28	27.75	74.00
S4.29	27.25	73.50

Based on Table 4, all S4 lines that have a tolerance level in the tolerant category can be determined for use in making S5 lines. Lines that are tolerant to drought stress are lines S4.7, S4.15 and S4.24. This is because the leaf angle of the three lines is less than 35^0 ranging from 26.50^0 to 28.75^0. The average harvest age of all lines is classified as super genjah ranging from 74.00 -75.50 days (<80 days).

Conclusion

The tolerance level of S4 lines to drought stress varies. Three S4 lines were tolerant to drought stress, namely lines S4.7, S4.15 and S4.24. Nine S4 lines have a fairly tolerant category, namely lines S4.1, S4.3, S4.8, S4.12, S4.13, S4.14, S4.21, S4.26 and S4.29; the rest are classified as sensitiveLines S4.7, S4.15 and S4.24 have tolerance levels classified as tolerant. Nine S4 lines have moderately tolerant levels classified and the rest are classified as sensitive. The average flowering, harvest age, yield components and average yield of S4 lines differ between drought stress conditions and normal conditions; while the growth variables are the same between the two conditions. The yield have a high negative correlation with the ISC value; the weight of dry cobs harvested per plant has a moderate negative correlation and the diameter of the cob has a low negative correlation. Other characters are not correlated with the ISC value. The length of the cob and the weight of dry cobs harvested per plant have a high positive correlation with the yield; while other characters have a moderate and low positive correlation and some are not correlated with the results. S4 lines that have the tolerant categories to drought stress can be used to form S5 lines.

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Author Contributions

I. W. S., I. G. P. M. A., conceptualized the research idea, research method, and analyzed the data. I. W. S and D. R. A., guided the writing of the review and editing, supervised and validated the instruments used in the research

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Conflicts of Interest

The authors declare no conflict of interest.

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