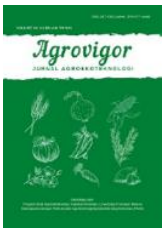
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Article

Selection of Maize Lines Resistant to Drought Stress Using Peg 6000 in The Vegetative Phase

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ABSTRACT

Selection of maize lines for drought stress is the initial step in developing maize varieties with high production and resistance to drought stress. The research aims to determine the characteristics of maize lines in the vegetative phase and to select for resistance of maize lines in the vegetative phase using PEG 6000. The research used a Randomized Complete Block Design (RCBD), which was arranged factorially with three replications and two treatments. The number of treatments in this study was 40 treatment combinations, so there were 120 experimental units. Factor I was genotype, and factor II was the giving of PEG-6000 solution. All maize lines were planted in polybags measuring 20 x 20 cm. There are two levels of PEG 6000 solution treatment: 0% concentration (optimum condition) and 10% concentration, which is equivalent to -0.19 Mpa (drought stress condition) (Mexal et al., 1975). When the plants are 10 days after planting (DAP), drought stress is applied using a 10% concentration of PEG 6000 solution. The observed characters were plant height, root length, leaf area, leaf chlorophyll, plant wet weight, and plant dry weight. The research results showed that drought stress treatment mostly reduced the character values of plant height, root length, leaf area, leaf chlorophyll, plant wet weight, and plant dry weight. All tested lines showed significant differences in all tested characters against optimum condition treatments and drought stress. The lines resistant to drought stress were G4, G9, G11, G13, G14, G15, G16, G17, and G19.

INTRODUCTION

Maize (*Zea mays* L) is one of the important food crops in Indonesia after rice (Febriandaru & Saptadi, 2019). Maize production in Indonesia increases every year because the need for maize increases every year, but the increase in maize production cannot meet the need for maize in Indonesia. The need for maize in 2023 was 15.70 million tons, met from domestic production of 13.79 million tons and imports of 1.19 million tons (Prasetyo et al., 2024). The need for maize in Indonesia increases by 4.41% per year (Aldillah, 2018). The increase in demand for maize is due to the use of maize not only as a food ingredient, but also as a feed ingredient for livestock and industry (Fitriana et al., 2024).

Increasing maize production in Indonesia can be achieved by optimising suboptimal land use (Wahid et al., 2020). The vast area of suboptimal land in Indonesia is the main capital for increasing maize productivity. The area of suboptimal land in Indonesia is 189.2 million hectares, consisting of 108.8 million hectares of acidic land, 11 million hectares of tidal swamp land, 9.2 million hectares of lowland swamp land, 14.9 million hectares of peat land, and 13.3 million hectares of dry land (Mulyani & Sarwani, 2013). Dry land with a dry climate in Indonesia is spread across East Kalimantan, East Java, Bali, NTB, and NTT. Madura Island is one of the East Java areas with suboptimal land (dry land with a dry climate). Suboptimal land impacts maize productivity on Madura Island, which was very low (2.21 tons ha^{-1}) in 2020 (Dinas Pertanian Tanaman Pangan Holtikultura dan Perkebunan Bangkalan, 2021). Developing maize varieties with high production characteristics and resistance to drought stress is a strategic step to increase maize production on dry land with a dry climate (Amzeri et al., 2022).

The initial step in assembling superior varieties is to identify lines that are resistant to drought stress (Amzeri et al., 2024). These lines, which are resistant to drought stress, can be used as parents in the assembly of maize varieties with high production characteristics and resistance to drought stress. One method of simulating maize resistance under drought stress conditions is to use a solution of Poly Ethylene Glycol (PEG) 6000. This compound can produce osmotic pressure that lowers water potential without causing direct toxicity to plant tissues, thus allowing evaluation of plant responses to drought stress (Yang et al., 2021). Evaluation of drought stress using PEG 6000 in maize plants can be carried out at the germination and vegetative phases. In both phases, plants require a fairly high water supply. Water shortages during these phases impact the plant's overall growth and maize productivity (Song et al., 2019) (Lamlom et al., 2024)

One quantitative approach to assess plant resistance to drought is the Drought Sensitivity Index (DSI) (Yerzhebayeva et al., 2024). The drought sensitivity index was developed as a parameter that measures the relative response of a genotype to drought stress compared to the average of all genotypes tested (Fischer & Maurer, 1978). DSI values approaching zero indicate that the genotype is more resistant to drought because the yield reduction is relatively small. On the other hand, high DSI values indicate that the plant is very sensitive to drought stress. The research aims to determine the characteristics of maize lines in the vegetative phase and to select for resistance of maize lines in the vegetative phase using PEG 6000.

MATERIALS AND METHODS

Plant Materials and Research Implementation

The research was conducted in December 2024 - March 2025. The research was conducted in the experimental garden of the Agrotechnology Study Program, University of Trunojoyo Madura. The genetic material used in this research was 20 maize lines. The research used a Randomized Complete Block Design (RCBD), which was arranged factorially with three replications and two treatments. The number of treatments in this study was 40 treatment combinations, so there were 120 experimental units. Factor I was genotype, and factor II was the giving of PEG-6000 solution. All maize lines were planted in polybags measuring 20 x 20 cm. There are two levels of PEG 6000 solution treatment: 0% concentration (optimum condition) and 10% concentration, which is equivalent to -0.19 Mpa (drought stress condition) (Mexal et al., 1975). When the plants are 10 days after planting (DAP), drought stress is applied using a 10% concentration of PEG 6000 solution. The watering frequency was once every seven days until the plant was about to flower. The amount of water given was 50 ml, where the level of drought stress treatment was adjusted to the

concentration of the PEG 6000 solution that had been determined.

Data Collection and Analysis

The characters observed in this research were:

1. Plant height. Plant height is measured from the base of the plant stem to the highest leaf.
2. Root length. Root length is measured with a ruler from the base to the tip of the root.
3. Leaf area. Leaf area is measured using the length times width method.
4. Leaf chlorophyll value. Leaf chlorophyll value is measured by extracting it from leaf samples using acetone. Then, the chlorophyll is tested spectrophotometrically at each wavelength.
5. Plant wet weight. Weighing the plant when it is 40 DAP.
6. Plant dry weight. Weighing plants that have been air-dried for three days.
7. Drought Sensitivity Index (DSI), calculated based on the formula proposed by Fischer & Maurer (1978).

$$\text{DSI} = \frac{1 - (\frac{Yp}{Y})}{1 - (\frac{Xp}{X})}$$

Where: Yp: The average of a genotype under drought stress conditions, Y: The average of a genotype under optimum conditions, Xp: The average of all genotypes under drought stress conditions, X: The average of all genotypes under drought stress conditions.
The DSI value is used to determine the tolerance level to drought stress. If the DSI value is ≤0.5 (the genotype is tolerant to drought stress), the value is 0.5<DSI≤1.0 (the genotype is moderately tolerant), and the DSI value>1.0 (the genotype is drought sensitive).

Quantitative character data was analyzed using the F test. If there is a significant effect, continue with the Honestly Significant Difference (HSD) Test ($p<0.05$) using STAR 2.01 software

RESULTS AND DISCUSSION

Drought-tolerant maize lines can be evaluated directly based on the relative decrease in characters under drought conditions compared to optimum conditions (Zhang et al., 2024). The research results of the drought stress in the vegetative phase on 20 lines using PEG 6000 showed differences in response in all quantitative characters measured. The treatment of planting maize lines under optimum conditions had higher values for all measured characters (plant height, root length, leaf area, leaf chlorophyll, plant wet weight, and plant dry weight) compared to drought stress conditions. The optimum environment provides sufficient water availability to support plant physiological processes (photosynthesis, respiration, and nutrient absorption) (Firdaus et al., 2022). This condition supports plants' maximum expression of their genetic potential, resulting in optimal vegetative plant growth (Nicotra et al., 2025). Conversely, drought conditions cause plants to experience disruptions in physiological processes, limitations in nutrient absorption, and trigger the accumulation of stress compounds that can inhibit their vegetative growth (Zia et al., 2021).

The Effect of Drought Stress on Plant Height and Root Length

The observations of plant height and root length characters showed that all tested maize lines were significantly different in the optimum and drought-stressed conditions (10% PEG 6000) (Table 1; Figure 1). The character of plant height and optimum conditions showed that G1 had the highest value (98.73 cm) and G16 had the lowest value (54.60 cm). Under drought conditions, G6 had the highest plant height (76.10 cm), and G3 and G14 had the lowest plant heights (48.67 cm and 49.23 cm, respectively). The average difference in height between plants grown under optimum conditions and those affected by drought is 16.06 cm. These results showed that drought stress treatment decreased the average plant height of all tested maize lines by 16.06 cm. Drought stress affects all aspects of plant growth, including physiological and biochemical processes, and causes modifications in plant anatomy and morphology (Seleiman et al., 2021).

Roots are an essential part of plants that absorb water and nutrients from the soil (Anbarasan & Ramesh, 2021). Under optimum conditions, G8 and G12 had the

longest roots of 58.46 cm and 58.03 cm, respectively. G16 had the shortest roots (36.13 cm) under optimum conditions. Under drought conditions, G19 had the longest roots (51.61 cm) and G5 had the shortest roots (17.83 cm). These results show that drought stress treatment decreased the average root length of all tested maize lines by 15.51 cm. Root growth decreased due to the addition of PEG 6000 by 10% because PEG 6000 can bind water, so it is not available to plants. The more concentrated the PEG 6000 concentration given, the more ethylene sub-units bind water, thereby preventing water from entering the plant tissue, which makes it increasingly difficult for plant roots to absorb water, which results in plants experiencing drought stress (Mustamu et al., 2023).

The Effect of Drought Stress on Leaf Area and Leaf Chlorophyll

Drought stress affects the character of leaf area and leaf chlorophyll. The average decrease in leaf area characters of all tested maize lines was 55.32 cm2, and the average decrease in leaf chlorophyll characters of all tested maize lines was 2.12 (mg/l) (Table 2). Leaf area character in optimum condition, G7 had the highest leaf area (321.96 cm2), and G16 had the lowest leaf area (103.09 cm^2). G9 had the highest leaf area (237.13 cm^2) in drought stress conditions, while G15 and G16 had the lowest leaf area of 113.5 cm^2 and 105.05 cm2, respectively. Under optimum conditions, maize plants can develop leaves optimally, and leaf growth will be disrupted under drought stress conditions.

Table1. The effect of drought stress on plant height and root length

Genotype	Plant Height (cm)			Root Length (cm)		
	Optimum	Drought Stress	Difference	Optimum	Drought Stress	Difference
G1	98.73 a	74.43 a-c	24.30	43.80 b-f	24.41 ef	19.39
G2	78.23 a-e	56.77 a-d	21.46	42.15 c-g	25.76 ef	16.39
G3	61.23 de	48.67 d	12.56	36.62 g	18.88 gh	17.74
G4	67.13 c-e	58.67 a-d	8.46	42.11 c-g	25.22 ef	16.89
G5	69.53 c-e	58.67 a-d	10.86	37.11 fg	17.83 h	19.28
G6	80.53 a-d	76.10 a	4.43	47.15 bc	31.34 cd	15.81
G7	83.63 a-d	62.17 a-d	21.46	49.63 b	35.22 c	14.41
G8	89.40 a-c	56.00 a-d	33.40	58.46 a	46.58 ab	11.88
G9	73.27 b-e	58.47 a-d	14.80	41.77 c-g	34.06 c	7.71
G10	64.13 de	53.47 b-d	10.66	39.83 d-g	23.13 e-h	1670
G11	67.80 c-e	59.70 a-d	8.10	38.77 e-g	22.14 f-h	16.63
G12	90.47 a-c	49.23 d	4124	58.03 a	45.53 b	12.50
G13	69.10 c-e	53.07 b-d	16.03	39.38 d-g	25.44 ef	13.94
G14	64.73 de	51.93 d	12.80	39.55 d-g	23.63 e-g	15.92
G15	70.23 c-e	57.00 a-d	13.23	40.47 c-g	31.25 cd	9.22
G16	54.60 e	52.50 cd	2.10	36.13 g	18.44 gh	17.69
G17	84.17 a-d	70.50 a-d	13.67	46.21 b-d	27.75 de	18.46
G18	68.10 c-e	62.60 a-d	5.50	41.48 c-g	20.71 f-h	20.77
G19	95.77 ab	75.07 ab	20.70	58.61 a	51.61 a	7.00
G20	78.57 a-e	53.13 b-d	25.44	44.89 b-e	23.00 e-h	21.89
Average	75.46	59.40	16.06	44.10	2859	15.51

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% HSD test

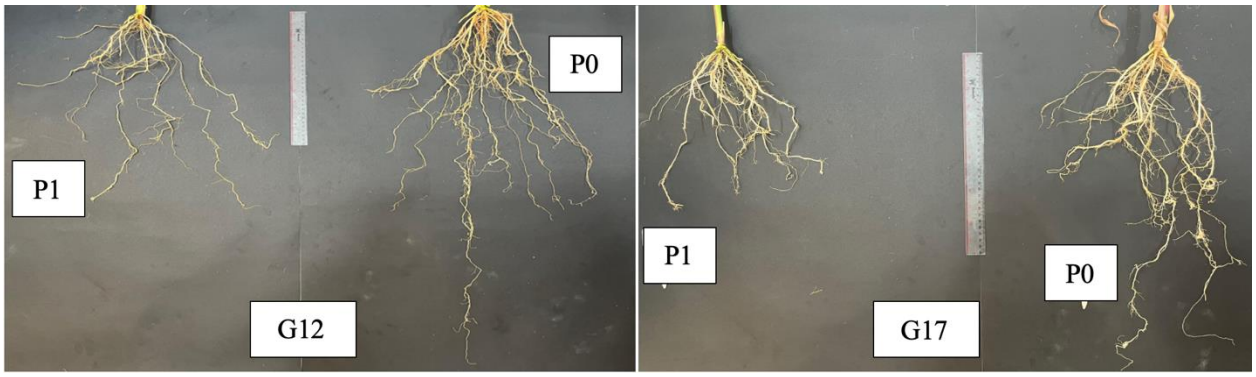


Figure 1. P0 = optimum condition (PEG 6000 = 0%), P1 = drought-stressed condition (PEG 6000 = 10%)

Table 2. Effect of drought stress on leaf area and leaf chlorophyll

Genotype	Leaf area (cm²)			Leaf chlorophyll (mg/l)		
	Optimum	Drought Stress	Difference	Optimum	Drought Stress	Difference
G1	160.32 gh	144.64 d-g	15.68	11.28 c	8.29 fg	2.99
G2	261.06 b-e	142.35 d-g	118.71	11.31 c	8.11 fg	3.20
G3	118.68 hi	115.42 g	3.26	11.37 c	7.94 gh	3.43
G4	250.76 c-e	196.21 a-c	54.55	11.11 cd	9.12 d-g	1.99
G5	252.91 c-e	204.80 a-c	48.11	11.15 cd	9.20 d-f	1.95
G6	267.22 b-d	183.63 b-d	83.59	11.22 cd	10.64 bc	0.58
G7	321.96 a	175.33 cd	146.63	10.16 d-f	5.74 i	4.42
G8	296.52 ab	160.86 c-f	135.66	11.21 cd	6.24 i	4.97
G9	249.59 c-e	237.13 a	12.46	11.12 cd	10.24 cd	0.88
G10	146.40 h	124.75 fg	21.65	11.53 c	10.71 bc	0.82
G11	200.67 fg	179.06 b-d	21.61	11.33 c	10.49 bc	0.84
G12	255.00 b-e	129.65 e-g	125.35	11.77 c	6.17 i	5.60
G13	275.64 bc	166.48 c-f	109.16	9.63 e-g	8.59 e-g	1.04
G14	224.66 ef	221.86 ab	2.80	9.09 fg	8.88 e-g	0.21
G15	118.70 hi	113.55 g	5.15	10.60 c-e	9.63 c-e	0.97
G16	103.09 i	105.15 g	-2.06	9.06 g	8.71 e-g	0.35
G17	276.58 bc	201.42 a-c	75.16	13.18 b	11.55 ab	1.63
G18	130.29 hi	126.86 fg	3.43	10.74 cd	9.72 c-e	1.02
G19	202.99 f	172.00 c-e	30.99	14.58 a	11.95 a	2.63
G20	233.57 d-f	138.93 d-g	94.64	12.91 b	6.83 hi	2.99
Average	217.33	162.00	55.32	11.21	8.93	2.12

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% HSD test

Water availability in the soil causes a decrease in cell turgor pressure which inhibits cell expansion and slows leaf growth (Ahluwalia et al., 2021). Leaf area under drought stress decreases because plants experiencing drought stress tend to regulate stomata opening to reduce water loss through transpiration (Yavas et al., 2024). The impact of this mechanism can help plants survive in drought-stressed conditions and have an impact on

reducing photosynthesis efficiency, which can inhibit leaf growth (Qiao et al., 2024).

One of drought stress's most obvious physiological impacts is a decrease in chlorophyll in leaf (Hu et al., 2023). Under optimum conditions, G19 had the highest leaf chlorophyll content (14.58 mg/l), and G16 had the lowest leaf chlorophyll content (9.06 mg/l). Drought stress disrupts the chlorophyll biosynthesis due to decreased activity of important enzymes such as chlorophyll synthase (Karami et al., 2025). Drought stress also causes increased production of reactive oxygen species (ROS), damaging chloroplast structure and accelerating chlorophyll degradation (Vijayaraghavareddy et al., 2022). Chlorophyll degradation causes leaves to experience chlorosis, which is characterised by the leaves turning yellow. The decrease in chlorophyll content impacts the decrease in the rate of photosynthesis. The decrease in the rate of photosynthesis results in a decrease in the accumulation of biomass produced by plants (Sun et al., 2024). Chlorophyll content can be used as an indicator that plants are resistant to drought stress. Plants that can maintain chlorophyll content or have low chlorophyll content in drought-stressed conditions can be used to indicate that the plant is resistant to drought stress. G14 is a maize line resistant to drought stress because the chlorophyll content was relatively stable under optimum conditions and drought stress. The decrease in chlorophyll G14 content was very low (0.21 mg/l) under drought conditions.

Table 3. Effect of drought stress on plant wet weight and plant dry weight

Genotype	Plant wet weight (g)			Plant dry weight (g)		
	Optimum	Drought Stress	Difference	Optimum	Drought Stress	Difference
G1	79.67 b-d	56.00 ab	23.67	23.00 a-c	19.00 ab	4.00
G2	81.00 b-d	50.00 b-d	31.00	25.33 ab	12.33 b-e	13.00
G3	44.33 g-j	37.67 c-f	6.66	15.33 bc	10.67 c-e	4.66
G4	41.33 h-j	37.00 c-f	4.33	16.00 bc	12.00 b-e	4.00
G5	62.67 d-g	37.00 c-f	25.67	23.00 a-c	13.67 b-e	9.33
G6	49.67 g-j	36.00 d-f	13.67	16.67 bc	9.33 de	7.34
G7	46.33 g-j	26.00 f	20.33	17.00 bc	7.67 e	9.33
G8	30.67 j	29.33 ef	1.34	14.33 bc	9.00 de	5.33
G9	87.67 bc	45.33 b-e	42.34	29.33 a	17.33 a-c	12.00
G10	79.33 b-e	35.67 d-f	43.66	29.67 a	9.67 c-e	20.00
G11	59.33 e-h	73.33 a	-14.00	22.67 a-c	23.33 a	-0.66
G12	92.33 ab	41.00 b-f	51.33	33.00 a	11.67 b-e	21.33
G13	52.33 f-i	41.33 b-f	11.00	16.33 bc	16.33 a-d	0.00
G14	70.33 c-f	36.33 d-f	34.00	25.00 a-c	15.00 b-e	10.00
G15	47.00 g-j	28.33 ef	18.67	14.67 bc	8.33 e	6.34
G16	38.33 ij	32.00 ef	6.33	14.00 c	9.67 c-e	4.33
G17	63.00 d-g	36.33 d-f	26.67	17.67 bc	11.67 b-e	6.00
G18	47.00 g-j	29.00 ef	18.00	14.67 bc	8.00 e	6.67
G19	109.00 a	54.33 bc	54.67	29.00 a	16.67 a-d	12.33
G20	54.00 f-i	55.67 b	-1.67	25.33 ab	12.33 b-e	13.00
Average	61.76	40.88	20.88	21.10	40.88	8.41

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% HSD test

Table 4. Average DSI values for all observed characters

Genotype	DSI						Index Average	Category
	PH	RL	LA	LC	PWW	PDW		
G1	1.16	1.26	0.38	1.30	0.88	0.44	0.90	MT
G2	1.29	1.11	1.79	1.39	1.13	1.29	1.33	DS
G3	0.96	1.38	0.11	1.48	0.44	0.76	0.86	MT
G4	0.59	1.14	0.85	0.88	0.31	0.63	0.73	MT
G5	0.73	1.48	0.75	0.86	1.21	1.02	1.01	DS
G6	0.26	0.95	1.23	0.25	0.81	1.10	0.77	MT
G7	1.21	0.83	1.79	2.14	1.30	1.38	1.44	DS
G8	1.76	0.58	1.80	2.18	0.13	0.93	1.23	DS
G9	0.95	0.52	0.20	0.39	1.43	1.03	0.75	MT
G10	0.78	1.19	0.58	0.35	1.63	1.69	1.04	DS
G11	0.56	1.22	0.42	0.36	-0.70	-0.07	0.30	T
G12	2.14	0.61	1.93	2.34	1.64	1.62	1.71	DS
G13	1.09	1.01	1.56	0.53	0.62	0.00	0.80	MT
G14	0.93	1.14	0.05	0.11	1.43	1.00	0.78	MT
G15	0.89	0.65	0.17	0.45	1.17	1.08	0.74	MT
G16	0.18	1.39	0.00	0.19	0.49	0.78	0.49	T
G17	0.76	1.14	1.07	0.61	1.25	0.85	0.95	MT
G18	0.38	1.42	0.10	0.47	1.13	1.14	0.77	MT
G19	1.02	0.34	0.60	0.89	1.48	1.07	0.90	MT
G20	1.52	1.39	1.59	2.32	-0.09	1.29	1.34	DS

Note: DSI = Drought Sensitivity Index, PH = Plant height, RL = root length, LA = leaf area, LC = leaf chlorophyll, PWW = Plant wet weight, PDW = Plant dry weight, T = tolerant, MT = moderately tolerant; DS = drought sensitive.

The Effect of Drought Stress on Plant Wet Weight and Plant Dry Weight

Drought stress affects the characteristics of plant wet weight and plant dry weight. The average decrease in wet plant weight of all tested maize lines was 20.88 g, and the average decrease in dry plant weight of all tested maize lines was 8.41 g (Table 3). Character of Plant wet weight in optimum conditions, G19 had the highest plant wet weight characteristics (109.00 g), and G8 had the lowest wet weight (30.67 g). G11 had the highest wet plant weight (73.33 g), and G7 had the lowest plant wet weight (26.00 g). The results of the research showed that drought stress conditions had an impact on reducing the plant wet weight. However, several tested maize lines showed that drought stress impacted increasing plant biomass characteristics, namely G11 and G20. One cause of the increased wet weight of plants under drought stress is the increased accumulation of osmotic compounds such as proline, soluble sugars, and certain ions synthesised by plants in response to drought (Panda et al., 2021). Accumulating these compounds can increase the mass of plant tissue, which causes an increase in the plant wet weight.

Under optimum conditions, G9, G10, and G11 had the highest plant dry weight, 29.33 g, 29.67 g, and 33.00 g, respectively, while G16 had the lowest dry weight (14.00 g). Under drought conditions, G11 had the highest plant dry weight (23.33 g), while G7, G15, and G18 had the lowest plant dry weight (7.67 g, 8.33 g, and 8.00 g, respectively). G11 experienced an increase in plant dry weight under drought stress conditions because the line also experienced an increase in plant dry weight under drought stress.

Determination of Maize Lines Resistant to Drought Stress

Drought Sensitivity Index (DSI) is a parameter for measuring plant tolerance to drought stress. DSI is used

to measure how much plant characteristics decrease in drought conditions compared to optimal (normal) conditions. The lower the DSI value of a plant character, the more tolerant the plant is to drought stress. Plant sensitivity to drought stress has three criteria, namely tolerant (DSI value ≤0.5), moderate (value 0.5<DSI≤1.0) and sensitive (DSI value>1.0). The results of the DSI calculations on six characters (plant height, root length, leaf area, leaf chlorophyll, plant wet weight, and plant dry weight) had different DSI values for each line tested (Table 4).

In plant height characters, lines tolerant to drought stress are G3, G4, G5, G6, G9, G10, G11, G14, G15, G17, and G18. Root length character, lines tolerant to drought stress are G6, G7, G8, G9, and G19. Leaf area character, lines that are tolerant to drought stress were G1, G3, G4, G5, G9, G10, G11, G14, G15, G16, G18, and G19. The leaf chlorophyll character, lines tolerant to drought stress were G4, G5, G6, G9, G10, G11, G13, G14, G15, G16, G17, G18, G19, and G20. The plant wet weight character, the lines tolerant to drought stress are G1, G3, G4, G6, G8, G11, and G16. Furthermore, the lines tolerant to drought stress in plant dry weight characters are G1, G3, G4G9, G11, G13, G16, and G17. Calculating the average DSI value of the six characters determines a line's tolerance to drought stress. The average character results showed that the lines tolerant to drought stress were G11 and G16. G4, G9, G13, G14, G15, G17, and G19 are the lines that were moderately tolerant to drought stress. The lines that were sensitive to drought stress were G1, G2, G3, G5, G6, G7, G8, G10, G12, and G20. Lines that are resistant to drought stress are determined by their tolerant and moderately tolerant categories, namely G4, G9, G11, G13, G14, G15, G16, G17, and G19.

CONCLUSION

Drought stress treatment mostly reduced the character values of plant height, root length, leaf area, leaf chlorophyll, plant wet weight, and plant dry weight. All tested lines showed significant differences in all tested characters against optimum condition treatments and drought stress. The lines resistant to drought stress were G4, G9, G11, G13, G14, G15, G16, G17, and G19.

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