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Identification of Drought Tolerance in Mutant Maize Genotypes to Ensure Raw Material Stability for the Food Industry

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ABSTRACT

Maize production stability in drought-prone environments is crucial to ensure continuous raw material supply for the food industry. This study aimed to evaluate the interaction between mutant maize genotypes and different water availability levels to identify drought-tolerant genotypes during the vegetative growth stage. The experiment was conducted in a screen house at the Faculty of Agriculture, Hasanuddin University, using a factorial split-plot design with three replications. Water availability levels (60%, 80%, and 100% of field capacity) were assigned as main plots, while six maize genotypes were arranged as subplots. Analysis of variance (ANOVA) revealed a highly significant interaction ($p \leq 0.01$) between genotype and water availability on root length, root volume, and flowering time. Under 60% field capacity, the Bisma 200 Gy genotype produced the longest root length (90.67 cm) and the highest root volume (116.67 mL), significantly exceeding the controls based on LSD at 1%. This genotype also showed lower leaf dryness and earlier male (51.94 days) and female (56.22 days) flowering. These findings demonstrate that Bisma 200 Gy exhibits enhanced drought tolerance through improved root development and accelerated flowering (drought escape), indicating strong potential as a stable maize raw material for water-limited environments.

INTRODUCTION

Maize (*Zea mays* L.) is a strategic commodity that plays a central role in Indonesia's national food security system, serving not only as a primary carbohydrate source but also as a key raw material for food processing and feed industries (Hadiyanto et al., 2021). In the context of food technology, a stable maize supply is essential to ensure continuous production of processed derivatives, including flour-based and functional food products (Aini et al., 2020). Therefore, efforts to increase maize productivity on marginal lands must simultaneously maintain agronomic performance and quality attributes to support industrial raw material stability (Suryadi et al., 2021).

Over the past five years, maize production stability in Indonesia has fluctuated considerably. National production declined from 22.59 million tons in 2019 to 22.29 million tons in 2020 and subsequently increased to 23.04 million tons in 2021, followed by further instability during 2022–2023 due to climate-related disturbances (BPS, 2024). Such fluctuations pose significant challenges to the food industry, as inconsistent raw material supply may disrupt production planning, increase processing costs, and reduce operational efficiency (Puspasari et al., 2023).

Weather anomalies associated with climate change, particularly irregular rainfall patterns, have become a major factor affecting maize productivity (Hadiyanto et al., 2021). Water deficits during the vegetative stage limit physiological performance by reducing cell turgor, photosynthetic activity, and biomass accumulation (Syafi'i et al., 2023). Prolonged drought stress may also accelerate leaf senescence and negatively affect kernel development, ultimately threatening yield stability and raw material quality for food processing (Fahri et al., 2023). Thus, developing drought-tolerant maize genotypes represents a strategic approach to maintaining production stability in water-limited environments.

Gamma-ray–induced mutagenesis has been widely applied to expand genetic variability and improve stress tolerance in crops. Previous research by Arifin (2012) identified irradiation doses of 100 Gy and 200 Gy as promising treatments for enhancing vegetative performance of Bisma and Lamuru varieties under water-stress conditions. However, earlier studies mainly emphasized general growth performance and did not comprehensively evaluate the interaction between mutant genotypes and graded water availability levels under controlled screening conditions. In particular, limited attention has been given to root morphological traits and flowering dynamics as key adaptive mechanisms during the vegetative phase. Furthermore, the relevance of these adaptive traits to ensuring stable maize raw material supply for the food industry remains insufficiently addressed.

Therefore, a systematic evaluation integrating genotype–water interaction and morphophysiological indicators is necessary to clarify the drought tolerance potential of mutant maize lines. This study aimed to (1) evaluate the interaction between mutant maize genotypes and different levels of water availability during the vegetative growth stage, (2) analyze root and flowering responses as indicators of drought adaptation, and (3) identify mutant genotypes with superior performance that may contribute to stable maize production in water-limited environments.

RESEARCH METHODS

The study was conducted at the Laboratory and Screen House of the Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar, from June to September 2026. During the experimental period, the average daytime temperature inside the greenhouse ranged from 28–34 °C. Watering treatments were applied daily according to the designated field capacity levels, and soil moisture was maintained through gravimetric monitoring to ensure consistency throughout the experiment. The primary genetic materials consisted of first-generation mutant (M1) maize seeds derived from Lamuru and Bisma varieties irradiated with gamma rays at doses of 100 Gy and 200 Gy. Non-irradiated Lamuru and Bisma (0 Gy) were used as control genotypes. Supporting materials included planting media, irrigation water, and NPK fertilizer applied according to standard agronomic recommendations.

The experiment was arranged in a factorial treatment structure using a split-plot design with three replications. Water availability levels were assigned as main plots, consisting of 60% (a1), 80% (a2), and 100% (a3) of field capacity. The subplots consisted of six maize genotypes: Bisma 100 Gy (k1), Bisma 200 Gy (k2), Lamuru 100 Gy (k3), Lamuru 200 Gy (k4), Bisma control (k5), and Lamuru control (k6). Each treatment combination consisted of two experimental units, resulting in a total of 108 experimental units.

Data were analyzed using analysis of variance (ANOVA) according to the split-plot design structure. Statistical calculations were performed using Microsoft Excel (Microsoft Corp., USA) with the Data Analysis ToolPak feature. When significant effects were detected ($p \leq 0.01$), mean comparisons were conducted using the Least Significant Difference (LSD) test at the 1% significance level.

Preparation of Growth Media and Planting Materials

The planting medium was prepared by removing interfering materials such as stones, residual roots, and large soil aggregates. The cleaned soil was then placed into containers (buckets) at a weight of 6 kg per unit, followed by water saturation to ensure optimal medium compaction.

Prior to planting, mutant maize seeds were pre-germinated in Petri dishes for approximately two days until radicle emergence was observed. Seeds exhibiting good germination vigor were subsequently transplanted into the planting medium at a density of two seeds per container.

Water Availability Regulation

Water availability levels were determined using a gravimetric weighing method based on field capacity (FC), following the formula described by Harun (2012).

$$KL\text{ku} = \frac{a-b}{b} x100\% \qquad KL\text{kl} = \frac{c-d}{d} x100\%$$

$$CTkl = w\ x\ \frac{(100+KLkl)}{(100+KLku)} \qquad Vol = CTkl - w$$

Planting and Crop Management

Planting was carried out after the growth medium had reached structural stability following water saturation performed one day prior. Pre-germinated maize seeds exhibiting radicle emergence and adequate vigor were manually transplanted into the planting containers. Two seeds were planted per container at a uniform planting depth to ensure synchronized early growth.

Crop Maintenance Management

Crop maintenance activities were focused on maintaining stable growing conditions and ensuring accurate application of water stress treatments across all experimental units. Water availability was adjusted routinely using a daily gravimetric weighing method, in which the volume of water added was based on the amount of weight loss to maintain the designated field capacity levels of 100% (a3), 80% (a2), and 60% (a1). In addition to irrigation management, environmental sanitation was performed through manual weed removal by uprooting competing vegetation around the planting medium to minimize nutrient competition. Concurrently, periodic soil loosening was carried out to improve medium aeration, thereby facilitating optimal root system development of the mutant maize without physical soil constraints.

Observed Parameters

The response of mutant maize genotypes was evaluated based on several vegetative growth and phenological parameters, as described below:

1. **Plant Height (cm):** Plant height was measured vertically from the base of the stem at the surface of the growth medium to the highest growing point of the main stem.
2. **Number of Leaves (leaves):** This parameter was recorded by counting all fully expanded leaves on each individual plant.
3. **Root Volume (mL):** Root volume was determined using the water displacement method by immersing the entire fresh root system into a graduated cylinder containing a known volume of water. The increase in water volume was recorded as the root volume.
4. **Root Length (cm):** Root length was measured using a ruler from the root base to the tip of the longest root.
5. **Leaf Drought Severity (%):** This parameter was calculated to assess the impact of water deficit by comparing the number of leaves exhibiting necrosis (dry leaves) to the total number of leaves per plant. The percentage of leaf drought severity was calculated using the following formula:

$$Kekeringan\ Daun = \frac{Jumlah\ Daun\ Kering}{Jumlah\ Daun\ Keseluruhan} \text{X } 100\%$$

6. **Male Flowering Time at 50% (days):** This parameter was recorded as the number of days from planting until more than 50% of the plant population within an experimental unit began to produce tassels.

7. **Female Flowering Time at 50% (days):** This parameter was calculated as the number of days from planting until more than 50% of the plant population exhibited silk emergence, with a minimum silk length exceeding 2 cm.

RESULTS AND DISCUSSION

Plant Height

The results of the analysis indicated that maize genotype and water availability level had a highly significant effect on plant height. The highest mean plant height was observed in genotype k4 (Lamuru 200 Gy) under optimal water availability (a3), reaching 222.17 cm. The reduction in plant height with increasing water deficit reflects a physiological response associated with inhibited cell division and elongation in the apical meristem tissues (Fahri et al., 2023). Water availability at 60% (a1) caused a substantial decrease in cellular turgor pressure, thereby restricting leaf and stem expansion as a mechanism to improve water-use efficiency under drought conditions (Purwanto et al., 2022). Despite the decline in growth under severe water stress, genotypes k2 (Bisma 200 Gy) and k4 (Lamuru 100 Gy) were able to maintain relatively stable growth performance compared to the control varieties. This finding indicates the effectiveness of mutation induction in enhancing plant plasticity responses to extreme environmental conditions (Widiarsih et al., 2022).

Table 1. Mean Root Length (cm) of Maize Genotypes under Different Levels of Water Availability

Genotipe	Water Availability Level			Mean
	a_1	a_2	a_3	
k_1	149,17	181,50	175,67	168,78^a
k_2	140,83	164,17	172,17	159,06^c
k_3	139,33	155,33	180,33	158,33^c
k_4	155,00	177,33	222,17	184,33^c
k_5	119,67	163,50	184,67	155,94^c
k_6	139,50	161,17	195,17	165,28^{bc}
Mean	140,58^r	167,17^q	188,36^p	165,37

Note: - LSD values at α = 0.01 were 9,45 for genotype and 13.62 for water availability.
- Values followed by different letters in the same column (a–c) and row (p–r) indicate significant differences based on the LSD test at α = 0.01.

Stability of plant height during the vegetative phase is crucial in determining the plant's capacity to intercept solar radiation for photosynthesis. Genotypes capable of maintaining vigorous growth under marginal land conditions can ensure sufficient biomass accumulation, which ultimately influences kernel filling quality as a critical raw material for the food industry (Suryadi et al., 2021). This morphological adaptive capacity serves as an important indicator in the selection of mutant maize genotypes that are resilient to global climate uncertainty (Hadiyanto et al., 2021).

Number of Leaves

Based on the analysis of variance, genotype, water availability level, and their interaction had no significant effect on the number of leaves in maize plants. Although no statistically significant differences were detected, variations in leaf number were observed among treatment combinations, as illustrated in Figure 1. The highest mean number of leaves was recorded in the Bisma 200 Gy genotype (k2) under 60% water availability (a1), with an average of 15.6 leaves per plant.

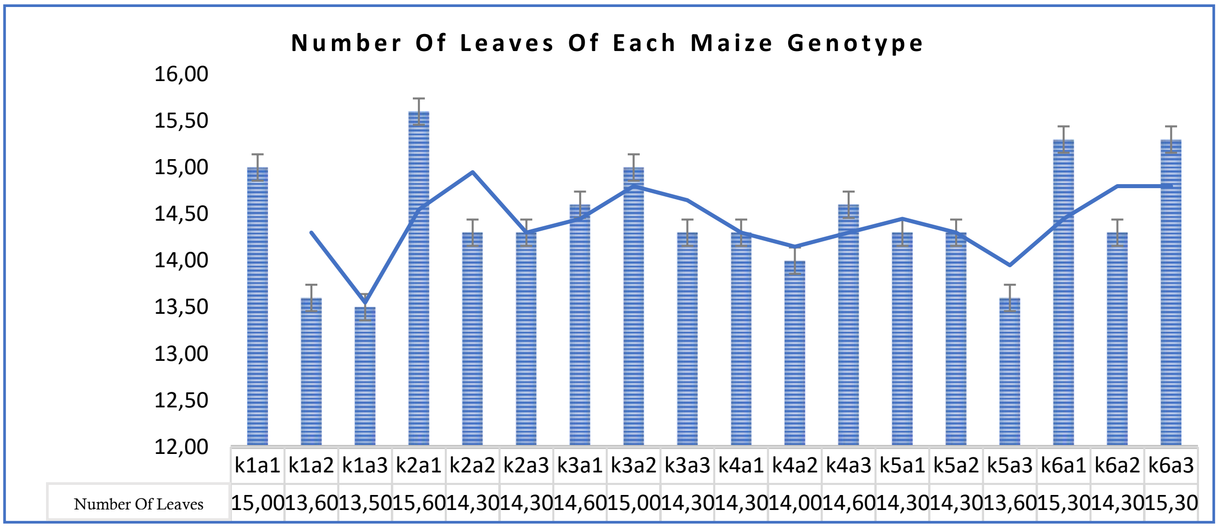

Figure 1. Mean number of leaves under different treatment combinations

The non-significant effect of water stress on leaf number indicates the stability of morphological traits in the evaluated genotypes, where leaf expression remained relatively constant despite exposure to varying environmental pressures (Syafi'i et al., 2023). Stability in leaf number under water-deficit conditions is particularly important, as leaves function as the primary organs of photosynthesis that ensure assimilate supply to support the quality of food-related yield components (Suryadi et al., 2021). The ability of plants to maintain photosynthetic surface area and cellular integrity through an optimal number of leaves serves as a key indicator of tolerance to abiotic stress in marginal lands (Muis et al., 2020). Ultimately, the efficiency of the canopy system contributes to the sustainability of food raw material production amid the challenges posed by global climate change (Hadiyanto et al., 2021).

Root Length and Root Volume

Based on the analysis of variance, the interaction between genotype and water availability exerted a highly significant effect on root length of maize plants. As presented in Table 2, the Bisma 200 Gy genotype (k2) consistently produced the longest average root length across all levels of water availability compared to the other genotypes. Under the most severe water stress condition (60% water availability; a1), Bisma 200 Gy (k2) exhibited root growth reaching 90.67 cm, which was significantly different from all other treatment interactions.

The significant increase in root length observed in mutant genotypes under water deficit conditions represents a crucial morphological adaptation that enables plants to explore and access water resources located in deeper layers of the growing medium (Li et al., 2024). The superiority of the Bisma 200 Gy genotype (k2) in extending its root system demonstrates that gamma-ray mutation induction at a dose of 200 Gy is effective in generating favorable genetic variability through improvements in root architecture, thereby enhancing drought tolerance (Widiarsih et al., 2022). Longer roots allow plants to maintain internal water status and cellular turgor pressure, ensuring that metabolic processes remain functional despite limited soil moisture availability (Hadiyanto et al., 2021). This massive and penetrative root system serves as a primary mechanism by which plants mitigate the adverse effects of drought, securing the continuity of the vegetative phase as a fundamental basis for food raw material production in dryland environments (Muis et al., 2020).

Table 2. Mean Root Length (cm) and Root Volume (ml) as Affected by the Interaction between Genotype and Water Availability Level

Genotipe	Root Length (cm)			Root Volume (ml)		
	Water Availability Level			Water Availability Level		
	a_1	a_2	a_3	a_1	a_2	a_3
k_1	$50{,}51^{b}_{p}$	$49{,}33^{c}_{p}$	$52{,}33^{c}_{p}$	$87{,}50^{b}_{r}$	$109{,}33^{b}_{q}$	$129{,}83^{b}_{p}$
k_2	$90{,}67^{a}_{p}$	$80{,}00^{a}_{p}$	$89{,}50^{a}_{p}$	$116{,}67^{a}_{r}$	$139{,}50^{a}_{q}$	$166{,}00^{a}_{p}$
k_3	$59{,}17^{b}_{p}$	$58{,}50^{bc}_{p}$	$62{,}00^{bc}_{p}$	$88{,}50^{b}_{p}$	$91{,}33^{c}_{p}$	$96{,}50^{c}_{p}$
k_4	$54{,}00^{b}_{p}$	$53{,}67^{bc}_{p}$	$61{,}33^{bc}_{p}$	$79{,}67^{bc}_{q}$	$73{,}33^{d}_{q}$	$139{,}17^{b}_{p}$
k_5	$55{,}83^{b}_{q}$	$75{,}00^{ab}_{p}$	$72{,}00^{b}_{p}$	$99{,}50^{ab}_{p}$	$106{,}67^{b}_{p}$	$106{,}83^{c}_{p}$
k_6	$61{,}00^{b}_{p}$	$63{,}83^{b}_{p}$	$64{,}33^{bc}_{q}$	$74{,}17^{c}_{q}$	$84{,}33^{cd}_{p}$	$75{,}33^{d}_{q}$

Note: - LSD values root length at α = 0.01 were 13.19 for genotype and 12.31 for water availability.
- LSD values root volume at α = 0.01 were 8.60 for genotype and 12.86 for water availability.
- Values followed by different letters in the same column (a–c) and row (p–r) indicate significant differences based on the LSD test at α = 0.01.

Based on the analysis of variance, the interaction between treatments had a highly significant effect on root volume. Under severe water stress conditions (60%), the Bisma 200 Gy genotype (k2) exhibited superior performance by producing the highest root volume, reaching 116.67 ml. The consistent performance of genotype k2 was also evident at water availability levels of 80% (a2) and 100% (a3), which resulted in the highest root volumes of 139.50 ml and 166.00 ml, respectively, compared to the other genotypes.

The increase in root volume observed in these mutant genotypes represents an adaptive response aimed at expanding the absorptive surface area to extract soil moisture more efficiently under marginal environmental conditions (Syafi'i et al., 2023). Gamma-ray–induced mutation has been shown to be effective in modifying the physical capacity of roots to withstand abiotic stress through alterations in genetic expression governing root morphology (Widiarsih et al., 2022). The enhancement of root biomass serves as a key mechanism for maintaining stable leaf water potential, as a more extensive root system enables plants to access deeper water sources when surface soil moisture becomes limited (Li et al., 2024). This phenomenon is closely associated with root morphological plasticity triggered by point mutations within the plant genome, which facilitate increases in root diameter and lateral branching, thereby improving hydraulic conductivity (Gomez et al., 2023). The altered root architecture observed in the Bisma 200 Gy genotype reflects an optimized allocation of biomass to belowground organs (root-to-shoot ratio) as a defensive strategy against extreme hydraulic deficit conditions (Zhang et al., 2022).

Leaf Dryness, Male Flowering Time, and Female Flowering Time

Based on the analysis of variance, genotype and water availability levels had a significant effect on the percentage of leaf dryness in maize, whereas the interaction between these two factors showed no significant effect. As presented in Table 3, the Bisma 200 Gy genotype (k2) exhibited the lowest average leaf dryness percentage at 35.31%, which was significantly different from most other genotypes, except for the Lamuru control (k6). In terms of water treatments, the 60% water availability level (a1) resulted in the lowest average leaf dryness value, reaching 38.16%.

Table 3. Leaf Dryness (%), Female Flowering Time (Days), and Male Flowering Time (Days) of Maize Genotypes under Different Water Availability Levels

Genotipe	Leaf Dryness (%)			Mean	Female Flowering Time (Days)			Mean	Male Flowering Time (Days)			Mean
	Water Availability Level				Water Availability Level				Water Availability Level			
	a_1	a_2	a_3		a_1	a_2	a_3		a_1	a_2	a_3	
k_1	39,55	44,76	43,67	$42{,}66^{b}$	55,00	51,67	49,50	$52{,}06^{b}$	61,33	58,00	54,67	$58{,}00^{b}$
k_2	32,36	36,21	37,36	$35{,}31^{a}$	54,17	50,50	51,17	$51{,}94^{b}$	57,67	55,67	55,33	$56{,}22^{b}$
k_3	42,84	47,20	46,23	$45{,}42^{b}$	55,83	53,00	49,17	$52{,}67^{ab}$	63,00	58,67	53,33	$58{,}33^{b}$
k_4	38,57	42,36	54,60	$45{,}18^{b}$	54,83	53,33	50,50	$52{,}89^{ab}$	59,67	59,67	53,33	$57{,}56^{b}$
k_5	40,14	42,64	48,18	$43{,}65^{b}$	53,67	52,17	50,83	$52{,}22^{ab}$	62,67	59,33	56,67	$59{,}56^{ab}$
k_6	35,49	38,45	50,00	$41{,}3^{a}$	56,83	55,00	52,33	$54{,}72^{a}$	67,00	63,33	58,67	$63{,}00^{a}$
Mean	$38{,}16^{p}$	$41{,}94^{pq}$	$46{,}67^{q}$		$55{,}05^{p}$	$52{,}61^{pq}$	$50{,}58^{q}$		$61{,}89^{p}$	$59{,}11^{p}$	$55{,}33^{q}$	

Note: - LSD values Leaf Dryness *at $\alpha = 0.01$ were 7.13 for genotype and 5.23 for water availability.*
- *LSD* Female Flowering Time *at $\alpha = 0.01$ were 2.57 for genotype and 2.45 for water availability.*
- *LSD* Male Flowering Time *at $\alpha = 0.01$ were 3.87 for genotype and 2.85 for water availability*
- *Values followed by different letters in the same column (a–c) and row (p–r) indicate significant differences based on the LSD test at $\alpha = 0.01$.*

The low percentage of leaf dryness observed in the Bisma 200 Gy mutant genotype (k2) serves as a strong indicator of enhanced cellular integrity and effective physiological tolerance mechanisms under water deficit conditions (Fahri et al., 2023). This response is closely associated with the superior root system architecture of genotype (k2), characterized by greater root length and volume, which enables the maintenance of hydraulic conductivity and ensures a more stable water supply to the aerial parts even under limited water availability (Hidayat et al., 2022). Furthermore, the reduced incidence of leaf necrosis in this mutant genotype reflects the effectiveness of its internal antioxidant defense system in mitigating oxidative damage induced by reactive oxygen species during soil moisture stress (Saputra & Nurhasanah, 2021). The ability to preserve green leaf area (stay-green trait) in this mutant line is critically important for sustaining carbon assimilation processes, which ultimately support kernel development and grain quality as essential attributes of maize for food industry raw materials (Suryadi et al., 2021).

Based on the analysis of variance, both genotype and water availability level had a significant effect on male flowering time in maize. The LSD test results presented in Table 5 indicate that the Bisma 200 Gy genotype (k2) exhibited the earliest male flowering time, with an average of 51.94 days, which was significantly earlier than that of the other genotypes. Regarding water availability treatments, optimal water supply at 100% (a3) resulted in the shortest average male flowering time of 50.58 days, which was significantly different from the other water levels, except for the 80% treatment (a2). In contrast, severe water stress at the 60% level (a1) caused the most pronounced delay in male flowering, with an average flowering time extending to 55.06 days.

The delay in male flowering time under water deficit conditions represents a protective physiological response, allowing plants to slow the transition from the vegetative to the reproductive phase as an energy- and assimilate-saving strategy (Purwanto et al., 2022). This response suggests that drought stress suppresses the rate of floral meristem differentiation and reduces the activity of flowering-related hormones, thereby extending the duration to anthesis (Amin et al., 2021). Nevertheless, the ability of the Bisma 200 Gy mutant genotype (k2) to flower earlier than the control varieties indicates improved metabolic stability and more efficient nutrient translocation as a consequence of gamma-ray–induced mutation (Pandin et al., 2020). The early flowering trait observed in this mutant line constitutes an important agronomic advantage, as it enables the crop to escape terminal drought stress and reduces the risk of yield loss under water-limited conditions (Muis et al., 2020).

Based on the analysis of variance, both genotype and water availability level had a highly significant effect on female flowering time in maize. The LSD test results presented in Table 6 indicate that the Bisma 200 Gy genotype (k2) exhibited the earliest female flowering time, with an average of 56.22 days, which was significantly earlier than the Lamuru control genotype (k6), although it did not differ significantly from the other mutant genotypes. In terms of water availability, optimal water supply at the 100% level (a3) resulted in the shortest average female flowering time of 55.33 days, which was significantly different from the water stress treatments (a1 and a2). In contrast, severe water deficit at the 60% level (a1) caused the greatest delay in female flowering, with an average of 61.89 days.

The delay in silk emergence (silking) under water stress conditions represents a protective response that postpones the reproductive phase due to restricted silk elongation caused by reduced cellular turgor pressure (Fahri et al., 2023). This condition often leads to a widening of the anthesis–silking interval (ASI), which substantially increases the risk of pollination failure and subsequent reductions in kernel set (Purwanto et al., 2022). Nevertheless, the Bisma 200 Gy mutant genotype (k2) maintained an earlier flowering response even under adverse environmental conditions. This performance suggests improved assimilate allocation to reproductive organs and greater osmotic stability compared with the other genotypes evaluated (Saputra & Nurhasanah, 2021). Such phenological superiority enhances the potential of genotype (k2) as a reliable raw material source for the food industry in drought-prone regions, as it effectively minimizes the risk of reproductive sterility induced by water deficit stress (Suryadi et al., 2021).

CONCLUSION

This study demonstrated a highly significant interaction between mutant maize genotypes and water availability levels affecting root traits and flowering time. A water availability level of 60% of field capacity proved to be an effective screening condition for identifying drought-tolerant genotypes. Among the evaluated materials, the Bisma 200 Gy genotype consistently exhibited superior drought tolerance compared with other genotypes, including non-irradiated controls. This tolerance was supported by advantageous morphophysiological characteristics, namely enhanced root development, reduced leaf dryness, and accelerated flowering as a drought escape mechanism.

These findings indicate that Bisma 200 Gy has strong potential as a stable maize genotype for water-limited environments and may contribute to maintaining raw material supply for the food processing industry. However, further studies are recommended to evaluate the stability and heritability of these adaptive traits in subsequent generations (M2 and M3), to conduct multilocation field trials under diverse agroclimatic conditions, and to assess grain yield and quality parameters to confirm its agronomic and industrial feasibility at the production scale.

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