International Journal of Technology and Education Research

https://e-journal.citakonsultindo.or.id/index.php/IJETER

E-ISSN: 2986-0733

Genetic Variation Of Sorghum (*Sorghum bicolor* L.) At Several Colchicine Concentrations Based On Agronomic Characteristics

Marta Adam[1], Indriati Husain[2], Mayasari Yamin[3], Nurdin[4], Suyono Dude[5]

[1] Agrotechnology Alumni at Gorontalo State University. Gorontalo. Indonesia
[2, 3, 4, 5] Agrotechnology Lecturer at Gorontalo State University. Gorontalo. Indonesia

Corresponding Author: mayasariyamin@ung.ac.id

Abstract

Sorghum (Sorghum bicolor L.) is a potential alternative to rice due to its high nutritional value. Genetic improvement of sorghum can be supported by colchicine, which induces genetic variation. This study aimed to identify high-performing sorghum varieties, determine the most effective colchicine concentration for increasing genetic variation, identify agronomic traits with high polygenic variability, and evaluate the best variety–colchicine interaction based on agronomic traits. The study was conducted from January to April 2025 in the Greenhouse of the Agricultural Assembly and Modernization Agency, Gorontalo. A factorial Completely Randomized Design (CRD) was applied, consisting of two sorghum varieties (Numbu and Kawali) and four colchicine concentrations (0, 750, 1000, and 1250 ppm), with three replications, resulting in 24 experimental units. Results indicated that the Numbu variety showed the greatest genetic variability, particularly in leaf area. A colchicine concentration of 750 ppm was the most effective in enhancing variability among sorghum plants. Plant height, panicle length, and leaf area exhibited high genetic variation and were controlled by multiple genes. Furthermore, the interaction between Numbu and 750 ppm colchicine generated the most favorable agronomic performance, especially in panicle length, plant height, and leaf area. Therefore, this combination holds significant potential for colchicine-mediated sorghum improvement.

Keyword: Generative, Mutation, Potensial, Selection, Vegetative.

INTRODUCTION

Sorghum is a promising cereal crop that can serve as an alternative food source to rice. This crop exhibits superior water-use efficiency and strong drought tolerance (Putri., 2022). Per

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100 g, sorghum grain contains 73.0 g carbohydrates, 3.3 g fat, 11.0 g protein, 332 kcal energy, 28.0 mg calcium, 4.4 mg iron, 287 mg phosphorus, and 0.38 mg vitamin B1 (Mustika et al., 2019). indicating its potential for further development as a food source. In addition, sorghum is widely utilized as a high-quality livestock feed ingredient, making it valuable for the animal feed industry.

Over the last three years, Indonesia's sorghum output has seen significant changes. With a planting area of 4,355 hectares, sorghum production reached 15,243 tons in 2022. Spread throughout 17 provinces, production rose to 115,848 tons in 2023 with a planting area of 30,000 ha, and to 154,464 tons in 2024 with a planting area of 40,000 ha (Coordinating Ministry for Economic Affairs of the Republic of Indonesia, 2022). According to the Directorate of Cereal Cultivation (2019), Indonesia's sorghum output increased by a negligible amount, from 6,114 tons to 7,697 tons.

The increasing demand for sorghum consumption has not been matched by its production growth, despite its strong potential as an alternative food source in Indonesia. Sorghum output remains low and has not reached a significant market level (Pestarini & Wahyuningsih, 2017). To meet rising demand, production must be enhanced through effective cultivation practices and the use of superior genetic resources. As noted by Wulandari et al. (2019), a plant's traits are determined by its genetic potential, underscoring the need for appropriate variety development. Plant breeding serves as a key approach for generating improved sorghum varieties (Wahyuni et al., 2023), with mutation induction—particularly using colchicine—being one applicable technique.

Mutation is a change in genetic material occurring at either the gene or chromosomal level. This process may arise naturally or be induced through various methods. Artificial (induced) mutations can be generated using physical or chemical mutagens. Chemical induction may be carried out using colchicine, a compound that disrupts cell division and alters chromosome number. Colchicine is a chemical mutagen used to induce mutations. At optimal concentrations, it can produce polyploid plants with larger stem diameters compared to controls, although excessive concentrations or long exposure times may inhibit plant growth. Colchicine also increases genetic variation (Husain et al., 2022). Polyploidy refers to organisms with more than two chromosome sets (Arumingtyas., 2016). Genetic variation may also influence seed germination through physiological or biochemical mechanisms (Yamin et al., 2024).

Previous studies have shown that colchicine effectively induces significant phenotypic and genetic changes in various crops. Husain et al., (2022), reported that 0–0.5% colchicine affected tiller number, abnormal seed germination, and leaf color in M1 mutants of the Tajuk

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shallot variety. Qonitah et al., (2023), found that 900 ppm colchicine significantly influenced flowering time, fresh cob weight, cob diameter, row number, and total soluble solids in sweet corn. Harahap and Hakim., (2024), observed that a 12-hour soak in 50 ppm colchicine improved in vitro peanut embryo growth. Yamin et al., (2024), plant breeding can enhance genetic diversity, which can be assessed through the coefficient of genetic variation for each trait.

METHOD

The experiment was conducted from January 2025 to April 2025 in the Greenhouse of the Agricultural Assembly and Modernization Agency, Gorontalo Province, located at Jl. Van Gobel No. 270, Iloheluma, Tilongkabila, Bone Bolango Regency. The materials used included sorghum seeds of the Kawali and Numbu varieties, colchicine, NaOH, distilled water, 50 cm × 50 cm polybags, labels, soil, rice husks, and cow manure. The equipment used comprised 100 ml Erlenmeyer flasks, stirring rods, 600 ml bottles, shovels, buckets, digital scales, analytical balances, rulers/meters, calipers, a mobile phone camera, and writing instruments.

Factorial Completely Randomized Design (CRD) was employed in this study. The first factor was variety, consisting of two levels: V1 (Numbu) and V2 (Kawali). The second factor was colchicine concentration with four levels: K0 (Control), K1 (750 ppm), K2 (1000 ppm), and K3 (1250 ppm). The eight treatment combinations were replicated three times, resulting in 24 experimental units. Experimental procedures included preparing the planting media, preparing the colchicine solution, colchicine soaking, planting, watering, and weeding.

Field observation data were analyzed using heritability and genetic variability analyses, gene action estimation through skewness and kurtosis, and statistical applications including SAS, MINITAB, and Microsoft Excel. The evaluated traits included plant height (cm), leaf number, leaf length (cm), leaf width (cm), leaf area (cm^2), stem diameter (cm), number of open stomata, number of closed stomata, days to flowering, panicle length (cm), and root number.

RESULTS AND DISCUSSION

Analysis of Variance of Agronomic Traits in Sorghum Plants

The differences in mean squares (M0) for various agronomic traits with respect to varieties, colchicine, and their interaction are presented in Table 1. The mean squares for varieties in the observed traits showed a highly significant effect on leaf area. In contrast, the mean squares for colchicine showed no significant effect on all observed traits. Meanwhile, the mean squares for the interaction between varieties and colchicine revealed a significant effect on plant height, leaf area, and panicle length. This suggests that the observed traits were

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primarily influenced by the inherent characteristics of the varieties and their interaction rather than by the colchicine soaking treatment. Colchicine can inhibit seed germination and growth because it contains mutagenic compounds that are toxic to plant cells (Yulia et al., 2022).

Table 1. . Summary of Analysis of Variance for Various Agronomic Traits of Sorghum Varieties and Colchicine Concentration Treatments

Character	Mean Square			CV (%)
	Variety	Colchicine	V*C	
Vegetative				
Plant Height	1723,82ns	395,00 ns	1545,00*	19,38
Number of Leaves	0,38 ns	2,15 ns	1,82 ns	11,99
Leaf Length	151,00 ns	33,94 ns	51,21 ns	7,77
Leaf Width	5,42 ns	0,62 ns	0,99 ns	13,13
Leaf Area	56357,04**	1815,51 ns	13636,63*	10,45
Stem Diameter	0,14 ns	0,26 ns	0,34 ns	16,36
Open Stomata	748,17 ns	1291,67 ns	1875,61 ns	115,98
Closed Stomata	4845,04 ns	18347,82 ns	752,60 ns	99,06
Generative				
Flowering Age	104,17 ns	144,11 ns	188,72 ns	24,15
Panicle Length	23,80 ns	18,39 ns	114,08*	22,15
number of roots	204,17 ns	373,00 ns	356,94 ns	19,63

Note: **= significantly different at α = 5%; *= significantly different at α = 1%; ns = not significant

The coefficient of variation (CV) for the observed traits indicated a low value for leaf length (7.77%) and a high value for open stomata (115.98%). The low CV is suspected to be influenced by the genetics of the varieties and colchicine, resulting in relatively small variation in leaf length. In contrast, the high CV is influenced by the interaction between varietal genetics, colchicine, and the environment, leading to significant variation in open stomata. According to Wati et al., (2022), the coefficient of variation provides an indication of the diversity of the observed traits, where a smaller CV corresponds to a higher level of selection accuracy.

The observed traits indicated significant variation in leaf area, suggesting genetic differences between the varieties, which in turn affected this trait. This is consistent with Table 2, which presents the values for the Numbu and Kawali varieties.

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E-ISSN: 2986-0733

Table 2. Mean Performance of Two Sorghum Varieties for Leaf Area Trait

Variety	Parameters
	Leaf Area ($cm^{2)}$
Numbu Variety	683,31[a]
Kawali Variety	586,39[b]

Note: Numbers not followed by the same letter are significantly different at the 5% level

Table 2 shows that the leaf area of the Numbu and Kawali varieties differed significantly. The Numbu variety produced a higher leaf area, with a mean value of 683.31 cm^2, compared to the Kawali variety, which had a mean value of 586.39 cm^2. The difference in mean leaf area between the Numbu and Kawali varieties is influenced by the genetic factors inherent to each variety, resulting in variations that affect leaf growth and development. According to Subekti & Umar (2023), such differences are caused by both genetic and environmental factors, which influence the expression of traits within a plant population. Leaves are essential organs that produce assimilates for the plant. As the leaf area increases, the plant's capacity to capture light also increases, thereby enhancing the photosynthesis process.

The observed traits in Table 1 indicate that the mean square of the interaction between variety and colchicine had a significant effect on plant height, leaf area, and panicle length. Therefore, Table 3 is presented to examine the specific interaction between variety and colchicine.

Table 3. Summary of Plant Height, Leaf Area, and Panicle Length of Two Sorghum Varieties under Different Colchicine Concentrations

Treatment	Parameters		
Interaction	Plant Height (cm)	Leaf Area (cm^2)	Panic Length (cm)
Numbu Variety + Control	109,90[abc]	740,13[a]	13,83[c]
Numbu Variety + 750 ppm	114,17[abc]	632,80[abc]	27,00[a]
Numbu Variety + 1000 ppm	122,67[abc]	678,93[ab]	21,00[abc]
Numbu Variety + 1250 ppm	140,17[a]	681,37[ab]	18,83[abc]
Kawali Variety + Control	102,13[abc]	549,57[c]	23,50[ab]
Kawali Variety + 750 ppm	135,33[ab]	665,77[abc]	16,50[bc]
Kawali Variety + 1000 ppm	96,80[bc]	541,80[c]	24,50[ab]
Kawali Variety + 1250 ppm	84,83[c]	588,43[bc]	24,13[ab]

Note: Values not followed by the same letter are significantly different at the 5% level

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The observed trait of plant height showed different mean values between the two varieties. In the Numbu variety, the highest mean height was recorded at the 1250 ppm colchicine treatment (140.17 cm), while the lowest was at 750 ppm (114.17 cm), both lower than the control. Conversely, the Kawali variety showed its highest mean height at 750 ppm (135.33 cm) and the lowest at 1250 ppm (84.83 cm), compared with the control. The mean height of Numbu was lower than its varietal description (±187 cm), suggesting that plant height is strongly influenced by the genetic makeup of each variety. According to Gabesius et al., (2012), genetic differences among varieties contribute to phenotypic variation, as each variety expresses distinct traits. In contrast, the mean height of Kawali corresponded to its varietal description (±135 cm), indicating a positive response to colchicine treatment. Sinaga et al., (2014), reported that colchicine can influence plant height through polyploidization, a condition in which plants acquire additional chromosome sets. This increase in chromosome number may enhance the activity of genes regulating cellular metabolism, including protein synthesis, ultimately stimulating the production of growth-related hormones that support plant development.

The observed trait of leaf area showed different mean values between the two varieties. The Numbu variety produced the highest mean value at the colchicine concentration of 1250 ppm (681.37 cm^2) and the lowest at 750 ppm (632.80 cm^2) compared to the control. Meanwhile, the Kawali variety exhibited the highest mean value at 750 ppm (665.77 cm^2) and the lowest at 1000 ppm (541.80 cm^2) compared to the control. The leaf area trait displayed variable diversity, indicating that colchicine influenced leaf area through the induction of mutation. This is presumed to be due to chromosomal changes that carry traits affecting leaf area (Herman., 2013). Each plant exhibits a different response to colchicine treatment. Increasing the concentration of colchicine can result in a reduction in leaf area. As a toxic agent, colchicine can disrupt the mitotic process in cells. Mutations induced by colchicine not only produce changes in size and number but can also lead to a reduction in leaf size.

The observed trait of panicle length showed different mean values between the two varieties. The Numbu variety produced the highest mean value at a colchicine concentration of 750 ppm (27.00 cm) and the lowest at 1250 ppm (18.83 cm) compared to the control. Meanwhile, the Kawali variety showed the highest mean value at 1000 ppm (24.50 cm) and the lowest at 750 ppm (16.50 cm) compared to the control. Based on the results obtained, the Numbu variety exhibited panicle lengths within the range described for the variety (±22–23 cm), whereas the Kawali variety showed values below its varietal description (±28–29 cm). This suggests that colchicine influenced the panicle length of the Kawali variety by producing variations from the expected varietal description. An appropriate colchicine concentration can

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affect the observed traits. Colchicine can induce chromosome duplication in plants, thereby generating variability in specific traits (Yulia et al., 2022).

Genetic Diversity Analysis of Agronomic Traits in Sorghum Plants

Heritability is an estimate of gene action for a given trait. Determining its magnitude is essential, as it influences the success of selection. By understanding heritability values, breeders can design more effective and efficient strategies to improve target traits, thereby optimizing the breeding process and achieving selection goals. High heritability indicates that most of the observed variation is genetically controlled, meaning genetic factors contribute more than environmental influences. Traits with high heritability offer strong potential for developing genotypes with improved or desirable characteristics.

The coefficient of genetic variation (CGV) is used to determine whether the observed traits are influenced by genetic factors or environmental conditions. Variation caused by environmental factors is not heritable, whereas variation arising from genetic factors can be passed on to subsequent generations. According to Wati et al., (2022), the magnitude of variation within a population is determined by its genetic diversity, and broad variability indicates a strong basis for effective selection. The availability of wide genetic variation is essential for successful improvement through colchicine treatment. The values of variance components and heritability are presented in Table 4. Based on these data, high genetic variation and heritability support the selection of high-yielding M0 varieties under colchicine application. Therefore, genotypic variance partitioning in the analysis of variance was conducted to estimate the magnitude of genetic and environmental variance components.

Plant height, leaf length, leaf area, open and closed stomata, days to flowering, panicle length, and root number are agronomic variables that show significant environmental fluctuation. Plant height, leaf length, and leaf area are traits with a large genetic variance. Plant height, leaf length, leaf area, open and closed stomata, days to flowering, and root number are agronomic variables that exhibit substantial phenotypic variance. Traits with the most genetic variation can be subjected to selection pressure, which makes them appropriate for use as both selection criterion and a basis for selection (Yamin., 2014).

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Table 4. Variance Components, Heritability, and Genetic Variance Standard Deviation of Sorghum Agronomic Traits under Colchicine Concentrations

Character	σ^2e	σ^2g	σ^2p	Hbs		GCV	σ (σ^2G)
				Value	Criteria		
Vegetative							
Plant height	481,53	414,1	574,61	72,07	High	17,97	365,77
Number of leaves	2,54	0	0,85	0	Low	0	0,23
Leaf length	43,4	35,87	50,33	71,26	High	7,06	32,05
Leaf width	1,74	1,23	1,81	67,91	High	11,03	1,15
Leaf area	4405,21	17317,28	18785,68	92,18	High	20,73	11887,2
Stem diameter	0,27	0	0,09	0	Low	0	0,04
Open stomata	1363,08	0	454,36	0	Low	0	196,57
Closed stomata	9935,33	0	3311,78	0	Low	0	1332,1
Generative							
Flowering time	243,79	0	81,26	0	Low	0	30,37
Panicle length	21,97	0,61	7,93	7,73	Low	3,7	5,36
Number of roots	282,71	0	94,24	0	Low	19,63	49,44

Note: GCV = genetic coefficient of variation; σ^2e = environmental variance; σ^2g = genetic variance; σ^2p = phenotypic variance; h^2bs = broad-sense heritability; = standard deviation of genetic variance

The values of genetic variance and phenotypic variance can be used to estimate heritability. The range of heritability values for the observed characters was between 0 and 92.18%. Four characters exhibited high heritability values, namely plant height, leaf length, leaf width, and leaf area. The high broad-sense heritability is attributed to the high genetic variance for each character. Characters with high heritability indicate that the phenotypic variation in sorghum varieties is largely heritable to their progeny (Yamin., 2014). Jambormias et al., 2004), stated that high heritability values for quantitative traits suggest that the phenotypic variation observed in a generation predominantly reflects genetic variation that can be transmitted to the next generation.

The observed characters that fell into the low heritability category included leaf number (0), stem diameter (0), open stomata (0), closed stomata (0), flowering age (0), panicle length (7.73), and root number (0). This low heritability is suspected to be influenced by environmental factors affecting inheritance (Rostini et al., 2006). Jameela et al. (2014) noted that low heritability implies that these traits may change when grown in different environments.

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Conversely, characters exhibiting high heritability included plant height (72.07), leaf length (71.26), leaf width (67.91), and leaf area (92.18). High heritability values suggest that these traits display uniform expression and are predominantly determined by genetic factors rather than environmental influences (Satriawan et al., 2017). Yamin., (2014), also stated that high heritability indicates that the phenotypic variation in F3 families is largely heritable to subsequent generations. For agronomic characters with a heritability value of 0, this is due to their genetic variance being negative (-), which prevents unnecessary reduction in phenotypic variance (Yamin., 2014).

Estimation of Gene Action in the Agronomic Traits of Sorghum Plants

The variation in population traits and gene action is highly important as a basis for increasing the frequency of desirable genes through directed selection processes, whether through negative selection, positive selection, or a combination of both. This table is useful for estimating the number of genes and the type of gene action controlling a trait within a segregating population.

Skewness measures the asymmetry of a population distribution. A skewness value close to zero indicates that the trait is likely controlled by additive gene action. Negative skewness suggests additive gene action with duplicate epistasis, whereas positive skewness indicates additive gene action with complementary epistasis. Kurtosis describes the peakedness of the distribution. A kurtosis value > 3 indicates that the trait is governed by a few major genes, while a value < 3 suggests control by many genes (Sayurandi & Woelan, 2016). Additive gene action refers to the cumulative and independent contribution of each gene to the phenotype. Epistatic gene action occurs when one gene influences or masks the expression of another (Sihaloho et al., 2015). Duplicate epistasis reflects redundancy or similarity in gene effects, whereas complementary epistasis occurs when genes interact synergistically to produce a complete phenotypic expression.

Table 5. Skewness, Gene Action, Kurtosis Values, and Distribution Patterns of Agronomic Traits in Sorghum Plants

Agronomic Character	*Skewness*	Gene Action (Additive + Epistasis)	*Kurtosis*	Description
Plant height	0,064	Complementary	-0,884	Polygenic
Leaf area	-0,079	Duplicate	-1,105	Polygenic
Panicle length	-0,493	Duplicate	-0,825	Polygenic

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The variation observed in sorghum varieties is influenced not only by colchicine treatment and environmental factors but also by genetic factors, which can be inferred through gene action. Table 5 shows that two agronomic traits are influenced by additive gene action with duplicate epistasis effects, namely leaf area (-0.079) and panicle length (-0.493), and are controlled by multiple genes. Meanwhile, one agronomic trait, plant height (0.064), is influenced by additive gene action with complementary epistasis and is also controlled by multiple genes. This suggests the influence of the environment, variety × environment interactions, genes, and epistasis (Yamin et al., 2024). According to Insan & Wirnas., 2016), the presence of additive genes leads to similarities in the agronomic traits among varieties. Maryono et al., (2019), stated that kurtosis values indicate that the observed traits are controlled by multiple genes.

Direct Selection of Plant Height, Leaf Area, and Panicle Length in Two Sorghum Varieties under Colchicine Concentrations

Determining the time and mode of selection is based on an understanding of the genetic regulation of the target features. More genetic diversity is typically reflected in traits with high genetic variance and high heritability. Heritability determines the selection process based on individual phenotypic values (Jambormias et al., 2004). According to Jambormias et al., (2004), family selection is used when heredity is low and within-family variation is moderate, while individual selection is used when heritability is high and both within-family and between-family variance are substantial. Yamin., (2014) asserts that more selection progress is achieved when traits with high heritability, high genetic variance, and high genetic coefficient of variation are chosen. In this study, panicle length and leaf area were the selection variables used. Both traits were selected for at the same time, and variations for each trait were divided into four quadrants. A 90% confidence interval was used to establish the borders between the quadrants.

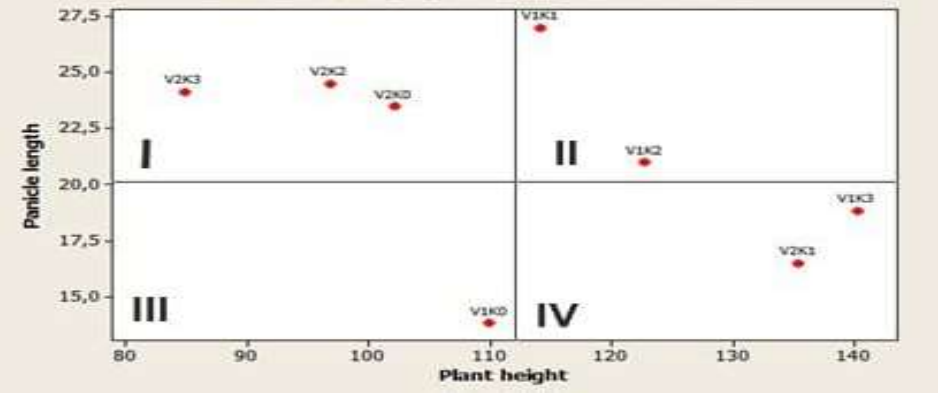


Figure 1. Distribution of Sorghum Varieties for Panicle Length and Plant Height Traits

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Figure 1 shows that Quadrant I represents varieties with high panicle length but low plant height. Quadrant II represents varieties with both high panicle length and high plant height. Quadrant III represents varieties with low panicle length and low plant height, while Quadrant IV represents varieties with low panicle length but high plant height.

Based on the quadrant separation of varieties and the applied colchicine concentrations, the potentially superior treatments are located in Quadrant II, specifically the Numbu variety treated with 1000 ppm colchicine and the Numbu variety treated with 750 ppm colchicine, where both panicle length and plant height are high. This suggests that plant height is positively and significantly correlated with panicle length, indicating that an increase in plant height is generally accompanied by an increase in panicle length (Alinnuha., 2018).

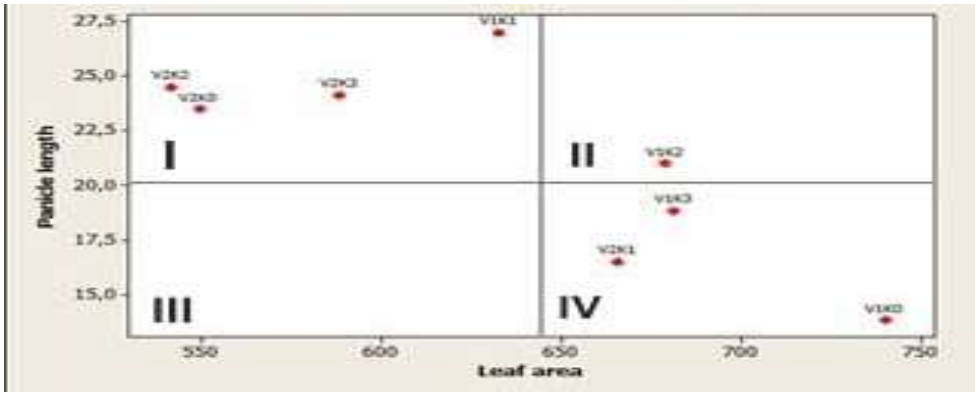


Figure 2. Distribution of Sorghum Varieties for Panicle Length and Leaf Area

Figure 2 shows that Quadrant I represents varieties with high panicle length but low leaf area. Quadrant II represents varieties with both high panicle length and high leaf area. Quadrant III represents varieties with low panicle length and low leaf area, while Quadrant IV represents varieties with low panicle length but high leaf area.

Based on the quadrant separation of varieties and the application of colchicine concentrations, the potentially advantageous treatments are located in Quadrant I, namely: Kawali variety without treatment, Kawali variety with 1000 ppm colchicine, Kawali variety with 1250 ppm colchicine, and Numbu variety with 750 ppm colchicine. In this quadrant, leaf area is narrow while panicle length is relatively long. It is suggested that narrower leaves result in lower transpiration, whereas longer panicles produce more seeds. According to Yamin., (2014), the separation in Quadrant III indicates better seed-filling ability compared to other families.

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CONCLUSION

Based on the results obtained, it can be concluded that: the Numbu variety exhibits the highest genetic variability based on leaf area; a colchicine concentration of 750 ppm is effective in enhancing the variability of sorghum plants; plant height, panicle length, and leaf area display high genetic variation and are controlled by multiple genes; and the interaction of the Numbu variety with 750 ppm colchicine shows the most favorable performance based on the selection traits of panicle length, plant height, and leaf area.

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