

# Mutant lines derived from Ethyl methanesulfonate treatment exhibit agronomic gain in Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet]

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## Research Article

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# Abstract

## Background

*Macrotyloma geocarpum* is a socio-economically important and nutrient-rich opportunity legume crop in which a broader genetic basis is required for an accelerated higher-yielding cultivar development.

## Materials and methods

Here, we reported for the first time the performance of a set of 300 mutants developed along with five local checks for their agronomic features spanning vegetative, physiological, developmental, seed and yield traits in an augmented design scheme. Descriptive statistics, correlation and multivariate analyses were combined to analyze the data.

## Results

Broader genotypic variability was observed in grain yield. Mutants were top outperformers for all traits. For instance, we observed a four-fold variation for grain yield between the best check (Check 3: 643.7 kg.ha<sup>-1</sup>) and the most productive mutant (ML645: 2,942.42 kg.ha<sup>-1</sup>) genotypes. Higher positive correlation ( $r = 0.98$ ,  $p < 0.001$ ) was recorded between the time to 50% flowering and time to maturity while higher negative one ( $r = -0.92$ ,  $p < 0.001$ ) was recorded between plant height and time to maturity. Heritability estimates ranged from 1.85 to 96.4% and two traits including seed length and grain yield exhibited high heritability estimates. The 305 genotypes were categorized in three clusters, with Cluster 3 encompassing individuals with the best characteristics.

## Conclusion

Genotypes of this Cluster 3 including for instance mutants ML645, ML619, ML10126 and ML1000 offer a good opportunity for market development and can be leveraged to advance elite individuals' selection for *M. geocarpum* improvement.

## Background

Orphan legumes offer significant nutritional value and resilience to abiotic stresses, making them a promising solution to address food and nutritional security amidst climate change challenges in sub-Saharan Africa [1–3]. Recently termed as “opportunity crops”, these underutilized legumes are crucial in developing countries, providing income for small-scale farmers and addressing micronutrient and protein deficiencies linked to calorie-dense diets from major crops prioritized by research and private sectors. Among these crops, Kersting's groundnut [(*Macrotyloma geocarpum* Harms) Maréchal &

Baudet] is a notable legume mostly cultivated in West African countries, especially in Benin, Togo and Ghana [4, 5].

Kersting's groundnut (KG) significantly contributes to socio-economic well-being through its diverse roles in nutrition, income generation, and soil fertility management. The crop is a rich source of protein, carbohydrates, and essential minerals like iron, zinc, calcium, and magnesium, making it valuable for human nutrition, especially in areas with limited access to animal protein [6, 7]. Its low-fat content and high arginine levels make it suitable for combating malnutrition in children through complementary food formulation [4]. Economically, the crop serves as a commercial commodity, generating substantial income for rural communities in countries like Benin, Togo, Ghana, and Burkina Faso. Its market value fluctuates seasonally, with the kilogram price ranging from 700 XOF to 3,500 XOF (USD 1.25 – USD 6.5), and peaking during periods of scarcity [8, 9]. Additionally, it holds medicinal properties that are valued in traditional healthcare [8, 10, 11], and also enhances soil fertility through nitrogen fixation, improving soil quality and structure [12]. Given its resilience to climate variability and prolonged drought, Kersting's groundnut is a promising candidate for sustainable cropping systems, reducing the intensity to chemical fertilizers' utilization.

Despite its potential, the production and utilization of Kersting's groundnut are limited by several constraints. Key production challenges included poor high seed yield, pests and diseases susceptibility, and limited access to improved varieties [13, 14]. These issues are compounded by the limited breeding initiatives aimed at developing high-performing cultivars. Farmers and end-users consistently expressed a preference for traits such as improved yield, early maturity, pest (viruses, fungi and pulse beetles' infestations) and disease resistance, tolerance to abiotic stress such as drought and high humidity and enhanced nutritional quality [4, 14]. Addressing these constraints requires a systematic approach to expand the genetic base of the crop.

Genetic variability is fundamental for the success of Kersting's groundnut breeding programs to enable the selection of superior traits aligned with farmers' and consumers' preferences [15]. Unfortunately, advances in cultivar development in *Macrotyloma geocarpum* was impeded by the lack of an appropriately diverse germplasm. Although increasing efforts are being made to gather as much diversity as possible through repetitive collecting missions, the intrinsic autogamous nature of *M. geocarpum* continued to keep a very narrow natural diversity in the species, which limits progress in breeding, as revealed by various genetic studies conducted these last years [16, 17]. To overcome this bottleneck, mutation breeding seems an alternative method to conventional plant breeding in the species as suggested by Coulibaly et al. [14] and a proven method for enhancing genetic variability [18]. Chemical mutagens such as ethyl methanesulfonate (EMS) have been widely used to induce point mutations, generating novel phenotypes for evaluation and selection in crop improvement programs [19].

Evaluating mutant lines for agromorphological traits is a critical step in identifying promising genotypes that outperform existing varieties or checks [20] as it allows breeders exploit useful variability for specific traits, advancing efforts to improve the crop's productivity and resilience. Thus, the present

study aimed to analyze the phenotypic diversity of *Kersting's groundnut* mutant lines derived from EMS treatment, with a focus on key agronomic traits. We hypothesized that there are mutant lines that outperformed local checks for key traits. This work provides foundational insights into the structure of variability among the mutant lines and their performance, offering valuable guidance for the genetic improvement of Kersting's groundnut.

## Materials and methods

### Study site

This experiment was conducted from May 15th 2022 to October 30th 2022 on the Seed services company experimental site in the district of Allada (latitude: 6.62; longitude: 2.06). During the course of the experiment, the total precipitation was 900.2 mm for an average temperature of 25.58, an average relative humidity of 90.55% and an average daily radiation of  $36.41 \text{ w.m}^{-2}$ . The soil is of sandy type (sand:  $768 \text{ g.kg}^{-1}$ ; clay:  $159 \text{ g.kg}^{-1}$  and silt:  $73 \text{ g.kg}^{-1}$ ) and characterized by a pH value of 6.

### Plant material and experimental design

A total of 305 genotypes including five check cultivars and 300 M2 mutant lines were used in this experiment. Checks cultivars were obtained from CalaviGen (the University of Abomey-Calavi genebank, republic of Benin), and included the black seed coat (BSC), red seed coat (RSC), mottle seed coat (MSC), white with red eye coat (WRC), and white (WSC) cultivars. Checks RSC, MSC and WRC were collected by the genebank in the locality of Manta (Longitude: 1.10, Latitude: 10.35), district of Boukoubé (Republic of Benin) while checks BSC and WSC were collected in Zahla (Longitude: 2.26, Latitude: 7.18), district of Zakpota still in the republic of Benin. These accessions are currently maintained by the genebank under the following accessions codes: HGU-MAG-001 (for BSC), HGU-MAG-012 (for RSC), HGU-MAG-010 (for MSC), HGU-MAG-011 (for WRC) and HGU-MAG-002 (for WSC). The 300 M2 mutant lines were generated by applying an ethyl methanesulfonate treatment at doses ranging from 500  $\mu\text{l}$  to 8500  $\mu\text{l}$  to five seed coat color-defined cultivars (868  $\mu\text{l}$  for BSc, 1698  $\mu\text{l}$  for RSC, 387 for  $\mu\text{l}$  WBC, 377 for  $\mu\text{l}$  WRC, 438  $\mu\text{l}$  for MSC). The number of mutants derived from each seed coat color type is presented in Table 1. Given the constraint imposed by the limited seeds available for the M2 mutants in development, the genotypes were laid out in an augmented design of 12 incomplete blocks of 30 genotypes each, the five checks being present in all incomplete block. Seeds of each genotype were sown on a 20 m long row plot and seeds holes were 0.5 m spaced. The trial was maintained clean throughout the experiment throughout a fortnight weeding of each plot.

Table 1  
Seed coat color of the mutant and check genotypes used in this experiment.

| Seed coat color            | Mutant     | Check       | Total      |
|----------------------------|------------|-------------|------------|
| Black (BSC)                | 1          | 1 (Check 1) | 2          |
| Mottle (MSC)               | 115        | 1 (Check 2) | 116        |
| Red (RSC)                  | 87         | 1 (Check 3) | 88         |
| White with black eye (WBC) | 26         | 0           | 27         |
| White with red eye (WRC)   | 71         | 1 (Check 5) | 72         |
| White (WSC)                |            | 1 (Check 6) | 1          |
| <b>Total</b>               | <b>300</b> | <b>5</b>    | <b>305</b> |

## Data collection

A total of 18 quantitative variable (Table 2) were included in this characterization. They were categorized in physiological parameters (chlorophyll content), vegetative growth parameters (e.g., plant height, plant canopy), development parameters (e.g., time to 50% flowering, time to podding, time to maturity), seed size traits (e.g., seed length, seed width, seed thickness), yield related traits (e.g., number of pods per plant, number of seeds per pod).

Table 2  
List of descriptors and methods of measurement.

| N° | Category      | Descriptor                | Code  | Methods of measurement                                                                                                                                         |
|----|---------------|---------------------------|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1  | Physiological | Chlorophyll content       | CHL   | Measured using SPAD chlorophyll meter on 10 randomly chosen plants and three randomly chosen leaves per plant                                                  |
| 2  | Vegetative    | Canopy                    | CAN   | Average of the plant spread taken in both the south-north and east-west directions, taken on 10 randomly chosen plants per plot                                |
| 3  |               | Plant height              | PLH   | From the soil surface to the plant tip using a tape measure on 10 randomly chosen plants                                                                       |
| 4  |               | Leaf width                | LEW   | Width of the broadest portion of the central leaflet taken, using a vernier caliper, on three leaflets per plant and on 10 randomly chosen plants on the plot  |
| 5  |               | Leaf length               | LEL   | Length of the broadest portion of the central leaflet taken, using a vernier caliper, on three leaflets per plant and on 10 randomly chosen plants on the plot |
| 6  |               | Petiole length            | PEL   | Measured on 10 plants per plot and on three leaves per plant from the base of petiole to beginning of limber                                                   |
| 7  | Development   | Time to first flowering   | T1FI  | Number of days from the sowing to the plant to flower                                                                                                          |
| 8  |               | Date to 50% flowering     | T50FI | Number of days from the sowing to 50% of plants in the treatment to flower                                                                                     |
| 9  |               | Time to podding           | Tpod  | Number of days from the sowing to the first pod bearing on the plot                                                                                            |
| 10 |               | Days to maturity          | Dmat  | Number of days from the sowing to the time were 50% of plants bore mature pod                                                                                  |
| 11 | Yield traits  | Number of pods per plant  | NPP   | Count of pods on each of 10 randomly chosen plant at the harvest time                                                                                          |
| 12 |               | Number of seeds per plant | NSP   | Count the number of seeds developed per plant on 10 plants randomly selected on each plot seed at the harvest time                                             |
| 13 |               | Number of seeds per pod   | NSPod | Count of seed in each of 10 randomly chosen pod at the harvest time                                                                                            |
| 14 |               | Grain yield               | GRY   | Product of average grain yield per plant at 10.5–11.5% water content                                                                                           |
| 15 |               | 100 seed weight           | 100SW | Average weight of ten samples of 100 seeds at 10.5–11.5% moisture content. Estimated value is validated at a coefficient of variation below 5%                 |

| N° | Category         | Descriptor     | Code | Methods of measurement                                                 |
|----|------------------|----------------|------|------------------------------------------------------------------------|
| 16 | Seed size traits | Seed width     | SEW  | Collected using a vernier caliper on 20 seeds randomly chosen per plot |
| 17 |                  | Seed length    | SEL  | Collected using a vernier caliper on 20 seeds randomly chosen per plot |
| 18 |                  | Seed thickness | SET  | Collected using a vernier caliper on 20 seeds randomly chosen per plot |

## Data analysis

Data were analyzed in the R environment [21]. Descriptive statistics including mean, minimum, maximum and coefficient of variation were used to assess the overall variation in the eighteen variables measured. Comparison of performance among the 305 materials was conducted for each variable using an analysis of variance throughout the function *augmentedRCBD* () implemented in the “augmentedRCBD” R package [22]. When treatment effect was significant, a genetic analysis based on parameters such as the phenotypic, genotypic and environmental variance, the broad sense heritability and genetic advance was reported. The correlation among tested variables was computed using the *ggcorrplot* () function of the “ggcorrplot” package [23]. To assess the relationship among the evaluated genotypes, a principal component analysis was first conducted using the *PCA* () function of the “FactoMineR” R package [24] to identify the most reasonable number of dimensions to retain. Then, a hierarchical clustering on the retained principal components analysis was conducted. The output of the clustering was then visualized using the *fviz\_clust* () function of the “factoextra” R package [25]

## Results

### Quantitative traits variation in the assessed germplasm

Descriptive statistics describing traits variation, adapted from the augmented design is presented in Table 3. Coefficients of variation ranged from 8.5–22.9%, 2.8–8.2%, 9.9–52%, 5.04 to 102%, for vegetative growth traits, development traits, yield traits and seed traits, respectively. Chlorophyll content, the only physiological parameter considered, had a coefficient variation of 6%. Although the highest coefficient of variation was observed for the seed thickness (CV: 102%), the yield-related traits were overall the most variable traits group while the development traits were the least variable one. Coefficients of variation for yield-related traits ranged from 9.88% (number of seeds per pod) to 55.2% (number of seeds per plant). Within the development traits, time to first flowering exhibited the largest variability (CV: 8.21%) while the day to maturity showed the lowest variability (CV: 2.1%). Canopy was the most variable vegetative growth traits (CV: 22.9%) while leaf width was the least variable one (CV: 8.5%) traits. Likewise, seed width was the least variable seed traits (CV: 5.04%) while the seed thickness exhibited the highest variability (102.2%) when it comes to seed traits. The assessed genotypes flowered

within on average 52 to 79 days and completed their cycle between 115 and 140 days. On average, 100 seeds weight ranged from 6.01 to 24.1 g.

Table 3

Quantitative variation of *Macrotyloma geocarpum* growth, physiological, seed and yield-related traits. Min: minimum; Max: Maximum; Std: Standard deviation; CV: Coefficient of variation.

| Trait                          | Code (unit)                | Min   | Max    | Mean  | Std   | CV (%) |
|--------------------------------|----------------------------|-------|--------|-------|-------|--------|
| Chlorophyll content            | CHL (SPAD)                 | 224.3 | 415.8  | 308.5 | 26.4  | 6.0    |
| Canopy width                   | CAN (cm)                   | 15.1  | 52.7   | 35.8  | 6.4   | 22.9   |
| Plant height                   | PLH (cm)                   | 14.9  | 39.0   | 25.6  | 4.1   | 11.2   |
| Lead width                     | LEW (cm)                   | 3.0   | 9.9    | 4.0   | 0.5   | 8.5    |
| Leaf length                    | LEL (cm)                   | 4.1   | 8.2    | 6.    | 0.7   | 14.1   |
| Petiole length                 | PEL (cm)                   | 7.9   | 33.1   | 17.6  | 3.7   | 15.2   |
| Time to first flowering (days) | T1FI (days)                | 48.63 | 77.23  | 59.34 | 5.19  | 8.2    |
| Time to 50% flowering(days)    | T50FI (days)               | 51.9  | 78.48  | 65.15 | 4.75  | 5.7    |
| Time to podding                | Tpod (days)                | 67.9  | 108.53 | 92.03 | 6.62  | 4.5    |
| Time to maturity               | Tmat (days)                | 115.3 | 140.3  | 129.3 | 4.6   | 2.8    |
| Number of pods per plant       | NPP                        | 5     | 227.3  | 65.9  | 38.1  | 51.1   |
| Number of seed per plant       | NSP                        | 8.5   | 309.7  | 85.1  | 49.9  | 55.2   |
| Number of seeds per pod        | NSPod                      | 0.9   | 1.9    | 1.29  | 0.2   | 9.9    |
| Grain yield                    | GRY (kg.ha <sup>-1</sup> ) | 41.1  | 2942.4 | 458.5 | 323.6 | 50.3   |
| Hundred seeds weight           | X100_SW (g)                | 6.01  | 24.12  | 9.7   | 1.95  | 16.1   |
| Seed width                     | SEW (mm)                   | 3.6   | 10.0   | 5.2   | 0.5   | 5.0    |
| Seed length                    | SEL (mm)                   | 5.1   | 76.6   | 7.7   | 4.0   | 10.3   |
| Seed thickness                 | SET (mm)                   | 2.2   | 39.5   | 3.9   | 2.4   | 102.2  |

## Variation of performance among genotypes

### Vegetative growth and physiological traits

The overall adjusted average plant height, canopy width, leaf width, leaf length, petiole length and chlorophyll content in the 305 genotypes were 25.61 cm, 35.81 cm, 4.04 cm, 6.02 cm and 17.57 cm, 308.4 SPAD Unit, respectively. The analysis of variance (Table 4) indicated that none of the variation

sources affected the leaf length ( $p > 0.05$ ). In opposite, all the variation sources affected the plant height ( $p < 0.01$ ). Out of the Checks, the Check 2 (Mottle-seeded cultivar) presented the highest plant height (30.86 cm) whereas the Check 5 (White with red eye cultivar) presented the lowest plant height (24.05 cm). Among the mutant lines, the mutant ML971 presented the shortest plants (14.96 cm) whereas ML898 showed the tallest ones (39.02 cm). Chlorophyll content also significantly varied among Checks in one hand and among mutant lines in the other hand, with checks overall presenting a higher pigment content than the mutant lines. The white-seeded cultivar check (Check 6: 326.73 SPAD Unit) and mutant ML1035 (415 SPAD Unit) had the highest chlorophyll pigment contents whereas the White with red eye cultivar check (Check 5: 285.69 SPAD unit) and ML582 (285.7) had the lowest chlorophyll pigment contents. All sources of variation except the block affected leaf width and petiole length. The broadest leaves in Checks and mutants were recorded in the white-seeded check (Check 6: 4.45 cm) and ML643 (9.86 cm), respectively, while the longest petioles in Checks and mutants were recorded in the mottle-seeded cultivar (Check 2: 20.74 cm) and ML992 (33.08 cm), respectively. As for the canopy, only the block ignoring the treatment and the treatment Check Vs Test exerted a significant difference, with checks group presenting a higher mean compared to mutant lines. Table 5 suggests that the top 10 performing genotypes for each of the six vegetative and physiological traits are only made up of mutant lines. This supported the existence of some mutants outperforming checks for all the six vegetative and physiological parameters.

Table 4

Analysis of variance for vegetative growth and physiological traits in *Macrotyloma geocarpum* genotypes. Values are Mean square<sup>significance level</sup>. Ns: not significant, \*\*: very significant and \*\*\*: highly significant.

| Source of variation                | Df  | PLH     | CAN                 | LEL                | LEW                | PEL                | CHL                 |
|------------------------------------|-----|---------|---------------------|--------------------|--------------------|--------------------|---------------------|
| Block (ignoring Treatments)        | 11  | 90.2**  | 352.7**             | 1.08 <sup>ns</sup> | 0.72**             | 91.2**             | 6244.8**            |
| Block (eliminating Treatments)     | 11  | 26.9**  | 116.8 <sup>ns</sup> | 0.82 <sup>ns</sup> | 0.16 <sup>ns</sup> | 11.1 <sup>ns</sup> | 1146.6**            |
| Treatment (ignoring Blocks)        | 304 | 17.3**  | 44.6 <sup>ns</sup>  | 0.31 <sup>ns</sup> | 0.29**             | 15.8**             | 674.2**             |
| Treatment (eliminating Blocks)     | 304 | 14.9*   | 36.1 <sup>ns</sup>  | 0.3 <sup>ns</sup>  | 0.27**             | 12.9 *             | 489.7 <sup>ns</sup> |
| Treatment: Check                   | 4   | 78.7**  | 66.0 <sup>ns</sup>  | 0.64 <sup>ns</sup> | 0.84**             | 60.5**             | 3936.7**            |
| Treatment: Test                    | 299 | 16.1 ** | 40.7 <sup>ns</sup>  | 0.3 <sup>ns</sup>  | 0.27**             | 15.2**             | 627.2**             |
| Treatment: Test vs. Check          | 1   | 110.3** | 1124.2**            | 1.75 <sup>ns</sup> | 1.8**              | 35.9*              | 1680.7*             |
| Treatment: Test and Test vs. Check | 300 | 14.12*  | 35.7 <sup>ns</sup>  | 0.29 <sup>ns</sup> | 0.26**             | 12.3*              | 443.7 <sup>ns</sup> |
| Residuals                          | 44  | 8.42    | 69.83               | 0.73               | 0.12               | 7.e3               | 345.6               |

Df: Degree of freedom, PLH: Plant height, CAN: Canopy width, LEL: Leaf length, PEL: Petiole length; CHL: Chlorophyll content.

Table 5  
Top 10 genotypes for each trait with significant variation among the *Macrotyloma geocarpum* genotypes.

| Traits                | Top ten genotypes out of the 305 assessed                                  |
|-----------------------|----------------------------------------------------------------------------|
| Pant height           | ML898, ML812, ML625, ML643, ML597, ML619, ML517, ML907, ML585, ML1045      |
| Canopy width          | ML678, ML619, ML643, ML554, ML529, ML507, ML511, ML812, ML898, ML720       |
| Leaf length           | ML1022, ML1011, ML1027, ML1021, ML626, ML619, ML779, ML1037, ML740, ML820  |
| Leaf width            | ML643, ML1022, ML1011, ML619, ML1021, ML820, ML1019, ML898, ML674, ML597   |
| Petiole length        | ML992, ML984, ML1026, ML898, ML1022, ML799, ML1011, ML838, ML585, ML674    |
| Chlorophyll content   | ML1035, ML1022, ML1021, ML1007, ML836, ML1037, ML850, ML917, ML896, ML1006 |
| Grain yield           | ML645, ML619, ML1026, ML1000, ML800, ML928, ML1021, ML924, ML1019, ML850   |
| Seed width            | ML626, ML619, ML522, ML548, ML845, ML1024, ML501, ML662, ML545, ML680      |
| Seed length           | ML827, ML522, ML845, ML545, ML1024, ML558, ML619, ML877, ML1026, ML548     |
| Time to 50% flowering | ML522, ML554, ML553, ML528, ML507, ML619, ML745, ML735, ML999, ML925       |
| Time to podding       | ML600, ML906, ML907, ML853, ML910, ML1037, ML949, ML1009, ML664, ML578     |

## Development traits

The average times to first flowering, 50% flowering, podding and seed maturation in the set of the 305 genotypes studied were  $59 \pm 5$  days,  $65 \pm 5$  days,  $92 \pm 5$  days, and  $129 \pm 5$  days, respectively. All these times varied significantly not only among checks, but also from the Check group to the test (mutant lines) group. Check 2 (Mottle seeded cultivar) consistently outperformed all other checks as the earliest maturing Check material with first flowering, 50% flowering, podding and seed maturing occurring 54, 58, 87 and 124 days after sowing, respectively. Check 6 (white seeded cultivar) was the latest maturing Check material with its seeds maturing after 139 days. The test group (mutant lines) was earlier maturing compared to the check group. For instance, podding occurred after on average 91 days in mutans lines while it happened only after 96 days in the checks. Among the mutant lines, significant to

very significant variations were also observed in all developmental times, except the time to first flowering. The earliest maturing mutant line was the ML522 in which first flowering, 50% flowering, podding and seed maturation occurred 50, 52, 81 and 115 days after sowing, respectively. Across the 305 genotypes, only the time to 50% flowering and the time to podding varied significantly (Table 6). The earliest flowering and podding genotypes were the mutant ML522 (52 days) and ML600 (64 days), respectively while the latest flowering and podding ones were ML959 (78 days) and ML978 (109 days), respectively. Only mutants formed the top ten earliest maturing genotypes out of the 305 genotypes (Table 5).

Table 6  
Analysis of variance for development traits in *Macrotyloma geocarpum* genotypes.  
Values are Mean square<sup>significance level</sup>. ns: not significant, \*\*: very significant and \*\*\*: highly significant. Df: Degree of freedom; T1F1: Time to first flowering; T50F1: Time to 50% percent flowering; Tmat: Time to maturity; Tpod: Time to podding.

| Source                             | Df  | T1F1               | T50F1              | Tmat               | Tpod               |
|------------------------------------|-----|--------------------|--------------------|--------------------|--------------------|
| Block (ignoring Treatments)        | 11  | 62.1 *             | 73.6 **            | 82 **              | 106.5 **           |
| Block (eliminating Treatments)     | 11  | 30.2 <sup>ns</sup> | 16.4 <sup>ns</sup> | 24.1 <sup>ns</sup> | 26.8 <sup>ns</sup> |
| Treatment (ignoring Blocks)        | 304 | 31.9 <sup>ns</sup> | 29.8 **            | 27.3 **            | 54.1 **            |
| Treatment (eliminating Blocks)     | 304 | 30.8 <sup>ns</sup> | 27.7 **            | 25.2 **            | 51.2 **            |
| Treatment: Check                   | 4   | 577.5 **           | 523.1 **           | 482.6 **           | 738.9 **           |
| Treatment: Test                    | 299 | 24.3 <sup>ns</sup> | 22.7 *             | 20.1 *             | 43.4 **            |
| Treatment: Test vs. Check          | 1   | 114.5 *            | 154.9 **           | 357.9 **           | 499.3 **           |
| Treatment: Test and Test vs. Check | 300 | 23.5 <sup>ns</sup> | 21.1 *             | 19.1 <sup>ns</sup> | 42 **              |
| Residuals                          | 44  | 23.9               | 13.84              | 13.31              | 17.55              |

## Seed traits

The average seed length, width and thickness were 7.72 mm, 5.24 mm and 3.04 mm, respectively. No source of variation exerted a significant effect on seed thickness (Table 7). Conversely seed length and seed width significantly varied, not only among checks but also among mutant lines ( $p < 0.05$ ). While the mottle-seeded cultivar check (Check 2: 7.82 mm) and the red seeded cultivar check (Check 3: 5.45 mm) exhibited the longest and the largest seeds, respectively, the white-seeded cultivar check (Check 6: 6.66 mm) and the white with red eye-seeded cultivar check (Check 5: 4.81 mm) rather presented the smallest and thinnest seeds, respectively. In mutant lines, genotypes ML857 (14 mm) and ML626 (10.03 mm) had the longest and largest seeds, respectively. In opposite genotypes ML512 (5.13 mm) and ML503 (3.57 mm) presented the lowest average values for seed length and seed width, respectively. In addition, Checks genotypes seed length was significantly higher than that of test genotypes (mutant lines).

Across all genotypes, seed length and seed width also significantly differed, with the top 10 performing genotypes for both traits only composed of mutant lines (Table 5).

Table 7  
Analysis of variance for seed traits in 305 *Macrotyloma geocarpum* genotypes. Values are Mean square<sup>significance level</sup>. ns: not significant, \*\*: very significant and \*\*\*: highly significant. Df: Degree of freedom; SEL: Seed Length; SEW: Seed width; SET: Seed thickness.

| Source                             | Df  | SEL                | SEW                | SET                    |
|------------------------------------|-----|--------------------|--------------------|------------------------|
| Block (ignoring Treatments)        | 11  | 15.12**            | 0.89**             | 4.67 <sup>ns</sup>     |
| Block (eliminating Treatments)     | 11  | 1.06 <sup>ns</sup> | 0.23**             | 14.89 <sup>ns</sup>    |
| Treatment (ignoring Blocks)        | 304 | 16.06**            | 0.27**             | 4.37 <sup>ns</sup>     |
| Treatment (eliminating Blocks)     | 304 | 15.55**            | 0.25**             | 4.74 <sup>ns</sup>     |
| Treatment: Check                   | 4   | 2.23 *             | 1.14**             | 12.89 <sup>ns</sup>    |
| Treatment: Test                    | 299 | 16.27**            | 0.26**             | 117613.5**             |
| Treatment: Test vs. Check          | 1   | 7.07**             | 0.27 <sup>ns</sup> | 3760.8 <sup>ns</sup>   |
| Treatment: Test and Test vs. Check | 300 | 15.73**            | 0.24**             | 101138.4 <sup>ns</sup> |
| Residuals                          | 44  | 0.63               | 0.07               | 52689.45               |

## Yield and related traits

*Macrotyloma geocarpum* produced on average 66 pods per plants and bear between 1 and 2 seeds per pod for an average number of 85 seeds per plant. On average 100 seeds weighed 9.68 g whereas the grain yield was 458 kg.ha<sup>-1</sup>. Apart from the block, no other source of variation exerted a significant effect on the number of pods per plant, number of seed per pod and the number of seeds per plant (Table 8). Conversely, the mass of 100 seeds significantly varied among Checks ( $p < 0.01$ ) with Check 2 (Mottle seeded cultivar) producing the heaviest seeds (10.70 g) while Check 6 produced the lightest seeds (7.91). Likewise, the seed yield differed not only among checks, but also among mutant lines as well as across genotypes. The highest yielding check was Check 3 (red-seeded cultivar) with an average yield of 643.7 kg.ha<sup>-1</sup> while the highest yielding mutant, ML645 (obtained from mottle-seeded cultivar), produced nearly four times more than the best check with an average yield of 2,942.42 kg.ha<sup>-1</sup>. Across the 305 genotypes tested, only mutants were ranked among the top 10 high yielding materials (Table 5) with their production ranging from 1,104.02 kg.ha<sup>-1</sup> to 2,942.42 kg.ha<sup>-1</sup>.

Table 8

Analysis of variance for yield and yield-related traits in *Macrotyloma geocarpum* genotypes. Values are Mean square<sup>significance level</sup>. ns: not significant, \*\*: very significant and \*\*\*: highly significant. NPP: Number of Pod per Plants; NSPod: Number of Seed per pod; NSP: Number of seed per plant; GRY: Seed yield; 100SW: Hundred seed weight.

| Source                             | NPP                   | NSPod              | NSP                   | GRY                    | 100SW              |
|------------------------------------|-----------------------|--------------------|-----------------------|------------------------|--------------------|
| Block (ignoring Treatments)        | 8967.04**             | 0.06**             | 17356.7**             | 490347.6**             | 2.61 <sup>ns</sup> |
| Block (eliminating Treatments)     | 873.47 <sup>ns</sup>  | 0.05**             | 2468.26 <sup>ns</sup> | 51376.08 <sup>ns</sup> | 3.15 <sup>ns</sup> |
| Treatment (ignoring Blocks)        | 1672.08 <sup>ns</sup> | 0.0 <sup>2ns</sup> | 2882.22 <sup>ns</sup> | 117697.51**            | 3.3 <sup>ns</sup>  |
| Treatment (eliminating Blocks)     | 1379.22 <sup>ns</sup> | 0.02 <sup>ns</sup> | 2343.5 <sup>ns</sup>  | 101813.68**            | 3.3 <sup>ns</sup>  |
| Treatment: Check                   | 1040.89 <sup>ns</sup> | 0.04 <sup>ns</sup> | 2318.2 <sup>ns</sup>  | 152461.02*             | 15.6**             |
| Treatment: Test                    | 1685.65 <sup>ns</sup> | 0.02 <sup>ns</sup> | 4.25 <sup>ns</sup>    | 0.26**                 | 3.1 <sup>ns</sup>  |
| Treatment: Test vs. Check          | 141.1 <sup>ns</sup>   | 0.01 <sup>ns</sup> | 4.66 <sup>ns</sup>    | 0.27 <sup>ns</sup>     | 3.7 <sup>ns</sup>  |
| Treatment: Test and Test vs. Check | 1383.73 <sup>ns</sup> | 0.02 <sup>ns</sup> | 4.63 <sup>ns</sup>    | 0.24**                 | 3.1 <sup>ns</sup>  |
| Residuals                          | 1138.71               | 0.02               | 15.39                 | 0.07                   | 2.43               |

## Correlation among studied traits

Observed correlations among the 18 traits studied ranged from – 0.92 to 0.98 (Fig. 1). Correlations among traits within the same group were overall positive, while negative correlations involved intergroup variables (e.g., development traits and grain yield). Correlations among development traits were the highest and varied between 0.91 (time to 50% flowering and time to podding) and 0.98 (Time to 50% flowering and Time to maturing). All correlations were significant among the vegetative traits, and the highest value ( $r = 0.88$ ) was observed between the plant height and the petiole length while the lowest correlation value ( $r = 0.64$ ) was between the plant height and the leaf length. There were also strong relationships among yield traits with the highest positive correlation ( $r = 0.98$ ) observed between the number of pods per plants and the number of seeds per plants, while the lowest correlation ( $r = 0.12$ ) was recorded between the number of seeds per plant and the number of seeds per pod. Correlations among seed traits were rather non-significant and ranged from – 0.03 to 0.18. The most positively correlated variable with the chlorophyll content was the seed width ( $r = 0.57$ ,  $p < 0.05$ ) while developmental traits were in general negatively correlated with the chlorophyll content ( $-0.58 < r < -0.52$ ,  $p < 0.05$ ), the vegetative growth traits ( $-0.92 < r < -0.57$ ,  $p < 0.05$ ), seed width ( $-0.43 < r < -0.41$ ,  $p < 0.05$ ) and seed thickness ( $-0.35 < r < -0.18$ ,  $p < 0.05$ ). Hundred seed weight was the most positively correlated trait with plant height ( $r = 0.53$ ,  $p < 0.05$ ) and the most negatively correlated with the time to 50% flowering ( $r = -0.64$ ,  $p < 0.05$ ). The top three most positively and significantly correlated vegetative traits with grain yield included the leaf length ( $r = 0.46$ ), petiole length ( $r = 0.42$ ) and leaf width ( $r = 0.41$ ).

# Relationships among studied genotypes

The principal component analysis (PCA) based on a total of 18 traits indicated that the cumulated variance explained by the first three components was 52% while that of the first five was nearly 70%. We consequently retained the first five components in our further analysis. The individual variance percentage explained by principal components (PCs) 1, 2, 3, 4 and 5 were 26.1%, 16.43%, 10.36%, 7.52% and 6.96%, respectively (Fig. 2). Dimension 1 was mostly positively correlated with plant height, petiole length and leaf width ( $r > 0.6$ ,  $p < 0.0001$ ) while Dimension 2 was so with time to 50% flowering and time to maturity ( $r > 0.6$ ,  $p < 0.0001$ ). On Dimension 3, was well represented but negatively the number of pods per plant ( $r = -0.6$ ,  $p < 0.0001$ ). Likewise, Dimensions 4 and 5 were positively and negatively correlated with seed length ( $r = 0.64$ ,  $p < 0.001$ ) and number of seeds per pod ( $r = -0.74$ ,  $p < 0.000$ ), respectively. Dimensions 1, 2, 3, 4 and 5 can be termed vegetative growth, development, grain yield, seed length and seed count dimensions, respectively.

CHL: Chlorophyll Content; GRY: Seed yield; Leaflet Length; LEW; Leaflet width; NPP: Number of Pod per Plants; NSP: Number of seed per plant; NSPod: Number of Seed per pod; PEL: Petiole length; PLH: Plant length; SEL: Seed Length; SET: Seed thickness; SEW: Seed width; T1F1: Time to first flowering; T50F1: Time to fifty percent flowering; Tmat: Time to maturity; Tpod: Time to podding ; X100\_SW: Hundred seed weight.

The hierarchical clustering applied on these five dimensions partitioned the 305 genotypes into three phenotypic groups (Fig. 3). Phenotypic groups 1, 2 and 3 were composed of 71, 108 and 126 genotypes, respectively. These represented 24%, 35% and 41%, respectively of the studied germplasm. A discriminative analysis revealed that these three groups demarcated from one another through eight key traits including the plant height, the petiole length, the chlorophyll content, the time to first flowering, the time to 50% flowering, the time to maturity, the seed width and the grain yield. A comparison of the three phenotypic groups based on these reduced number traits is presented in Fig. 4A-H. Basically, phenotypic group 1 was made up of late maturing genotypes with a poorer plant architecture compared with the overall performance of the whole germplasm studied. Genotypes in this group were on average  $23.02 \pm 0.47$  cm tall and took on average  $66 \pm 5$  days to flower and  $136 \pm 2$  days to mature. Their plants yielded on average  $399.5 \pm 26.48$  kg.ha<sup>-1</sup>. As for phenotypic group 2, it encompassed genotypes maturing quite earlier compared with the average germplasm but that performed poorly when it comes to other agronomic traits including plant architecture, seed traits and grain yield. Group 2 plants average height was  $23.6 \pm 0.3$  cm. They took on average  $57.69 \pm 1$  day to flower and  $127.63 \pm 1$  day to mature for an average yield of  $148.4 \pm 14$  kg.ha<sup>-1</sup>. Phenotypic group 3 was made up of the earliest maturing ( $57.26 \pm 1$  day for flowering and  $127.26 \pm 1$  day for maturing), most productive ( $648.28 \pm 33.43$  kg.ha<sup>-1</sup>) and architecturally noticeable genotypes (average plant height =  $28.57 \pm 0.29$  cm).

# Genetic analysis of studied traits

Genetic analyses only focused on the 15 traits for which the Treatment:check source of variation exhibited a significant effect. Broad sense heritability estimates ranged from to 1.8% (time to first flowering) to 96.4% (seed length) thus ranging from low to high. Four traits exhibited low heritability (1.8% – 25.1%), nine had moderate heritability estimate (32.45% -59.65%) while two presented a high estimated heritability (74.8% and 96.4%) (Fig. 5). The genetic advance over mean (GAM) also ranged from 0.31 to 103.68% and was categorized in three groups (Fig. 6). In total five traits exhibited high GAM with the seed length (103.68%), the grain yield (85.19%) and the number of pods per plant (41.76) being the most prominent. Three traits presented moderate GAM and included plant height (15.46%), leaf width (15.1%) and seed with (14.72%). The top three traits in the low GAM category included time to podding (8.8%), number of pods per plant (8.5% and Chlorophyl content (7.5%).

CAN: Canopy; CHL: Chlorophyl Content; GRY: Seed yield; Leaflet Length; LEW; Leaflet width; NPP: Number of Pod per Plants; NSP: Number of seed per plant; NSPod: Number of Seed per pod; PEL: Petiole length; PLH: Plant length; SEL: Seed Length; SET: Seed thickness; SEW: Seed width; T1F1: Time to first flowering; T50F1: Time to fifty percent flowering; Tmat: Time to maturity; Tpod: Time to podding ; X100\_SW: Hundred seed weight.

CAN: Canopy; CHL: Chlorophyl Content; GRY: Seed yield; Leaflet Length; LEW; Leaflet width; NPP: Number of Pod per Plants; NSP: Number of seed per plant; NSPod: Number of Seed per pod; PEL: Petiole length; PLH: Plant length; SEL: Seed Length; SET: Seed thickness; SEW: Seed width; T1F1: Time to first flowering; T50F1: Time to fifty percent flowering; Tmat: Time to maturity; Tpod: Time to podding ; X100\_SW: Hundred seed weight.

## Discussion

The present study reports the first phenotypic evaluation in Kersting's groundnut mutants marking a significant milestone in the genetic improvement of this underutilized crop. By inducing and characterizing mutations, this work provides a foundational platform for unlocking the genetic potential of Kersting's groundnut, which has long been neglected in mainstream breeding programs. The identification of variations among the mutant lines opens avenues for breeding programs aimed at enhancing yield, adaptability and other agronomic traits of economic importance in Kersting's groundnut.

## Quantitative traits variation

The coefficients of variation (CV) observed in this study provide prospective insights into the phenotypic diversity of Kersting's groundnut and highlight the transformative potential of mutagenesis in broadening its genetic base. Compared to previous studies [26, 27], which reported overall low genetic variability in natural populations of Kersting's groundnut, this study revealed significantly higher variability for several key traits, particularly yield-related and seed traits (Table 3). For instance, grain yield (CV: 50.2%), number of pods per plant (CV: 51.1%) and number of seeds per plant (CV: 55.2%) exhibited higher variability in

this study than in Akohoue et al. (2020, 2019), where CVs for these traits ranged from 34.8–42.4%. However, much greater variability was observed for grain yield by Coulibaly et al. [27] (CV: 49.1–64.1%). This could be due to genotype by environment interaction effects in the 5 environments of evaluation, the grain yield being a quantitative trait highly affected by environment in Kersting's groundnut [9]. Seed thickness showed an exceptionally high CV (102.2%) in this study, compared to much lower values recorded (4.38–7.1%) in previous studies [9, 16]. This suggests the presence of mutants with altered seed morphology and underscores the potential effects of EMS mutagenesis in inducing novel genetic variations, which could be useful in breeding program targeting improved seed quality.

In contrast, developmental traits such as time to 50% flowering (CV: 5.7%) and time to maturity (CV: 2.8%) were less variable, in line with earlier reports (CVs of 3.62–7.1%) [16, 27], suggesting their inherent stability across genotypes. The increased variability observed in this study, particularly for yield-related and seed traits, demonstrates the effectiveness of mutagenesis in overcoming the limited genetic diversity previously reported in Kersting's groundnut [13, 16, 17]. This expanded variability provides a valuable resource for breeding programs, enabling the selection of mutant lines with improved yield potential and desirable seed characteristics, paving the way for more efficient breeding strategies tailored to market needs.

The yield variation observed in the present study represents a significant breakthrough in the genetic improvement of Kersting's groundnut. The highest-yielding mutant line (ML645) produced an average yield of 2,942.42 kg.ha<sup>-1</sup>, nearly four times higher than the best-performing check (Check 3: 643.7 kg.ha<sup>-1</sup>). This increase in yield is unprecedented when compared to previous studies, which reported much lower yields. For instance, Coulibaly et al. [26] reported an average yield of 546.10 kg.ha<sup>-1</sup>, while Akohoué et al. [13] observed a yield range of 126.49 to 1,444.29 kg.ha<sup>-1</sup>. Similarly, Assogba et al. [8] reported yields ranging from 77 to 1,548 kg.ha<sup>-1</sup>, with the highest yield recorded for black-seeded cultivars under research station conditions. Even in studies focusing on genotype-by-environment interactions [27], yields ranged from 425.80 to 843.54 kg.ha<sup>-1</sup>, further highlighting the limited yield potential of existing materials. The high yields achieved by the mutant lines demonstrate that Kersting's groundnut has the potential to meet or even exceed the desired yield target of 1,500–2,000 kg.ha<sup>-1</sup> reported by Coulibaly et al. [14]. The results of this study, therefore, represent a substantial leap forward, with the top 10 high-yielding genotypes evaluated being exclusively mutants producing yields ranging from 1,104.02 to 2,942.42 kg.ha<sup>-1</sup> (Table 5), far exceeding the maximum yields previously reported. This mutant lines' performance can be attributed to the genetic variability induced by EMS mutagenesis. EMS has proven to induce useful mutations in mungbean [28], chickpea [29], cowpea [30], urdbean [31] and soybean [32]. Moreover, the outperformance recorded for the mutants in this study have been previously evidenced in lentil (*Lens culinaris* Medik.) [33] where authors used different concentrations of ethyl methanesulfonate (EMS) (0.10%, 0.25%, 0.50%, 0.75%, and 1.0%).

The highest yielding mutant in this study (ML645) exhibited a mottle-colored seed coat, whereas previous studies reported black-seeded [8] and white-seeded [14] cultivars among the most productive.

However, farmer preferences in Kersting's groundnut cultivation strongly favor white and white with black eye seed coat colors due to their higher market demand and consumer acceptance [8, 26]. While the mottle-colored ML645 mutant demonstrates outstanding yield potential, its adoption may be limited. Two major strategies can then be envisioned. First, future breeding efforts could focus on introgressing the high-yielding trait of ML645 into preferred seed coat color backgrounds, ensuring that improved varieties align with both agronomic performance and consumer demand. This approach would enhance the likelihood of farmer acceptance while leveraging the genetic gains achieved through mutagenesis. Second, based on all the significant traits observed, the mutant lines that consistently performed excellently included the ML619, ML1026, ML522, ML1021, ML1022, which were strikingly dominated by mottle-seeded cultivar derived mutants (4 out of four stable mutants). It may then become necessary to raise awareness on the potential of this mottle-seeded cultivar and embark the end-users on its acceptability.

In terms of correlation among traits, while the trends observed in this study overall corroborated previous findings [9], our study highlighted the time to first flowering, and not the time to 50% percent flowering [9], as the best development indicator of the plant yield. Likewise, the leaf length emerged as the most important vegetative traits correlate with the grain yield while the number of seed per plant remain the strongest positive yield component. The different associations offered the possibility to improve breeding efficiency through indirect and correlative selections.

## Clusters features and traits preference by end-users

Farmers preference in KG revolved around high yielding, drought tolerance, resistance against beetles [26], nutritional quality and any contributing factors such as for earliness that can help the plant escape abiotic stress [34]. In this study, the 305 materials were assessed for yield traits, earliness (development) traits, vegetative trait as well as physiological traits. From the hierarchical clustering analysis, only Cluster 3, representing 41% of the studied germplasm, comprises high-yielding genotypes that flower and mature early. Mutants in this cluster could be valuable for breeding programs aimed at improving yield and drought tolerance in Kersting's groundnut. In contrast, Cluster 1 consists of late-flowering and late-maturing genotypes with moderate yield ( $399.5 \text{ kg} \cdot \text{ha}^{-1}$ ), while Cluster 2 contains accessions that flower and mature early but exhibit very low grain yield ( $148.4 \text{ kg} \cdot \text{ha}^{-1}$ ), and therefore cannot be recommended, but could rather be conserved for depiction of unanticipated traits. The findings of this study interestingly expanded to chlorophyll activity the discriminatory traits among *M. geocarpum* phenotypic groups. Chlorophyll content is a key indicator of the plant photosynthetic activity, and plays a crucial role in the plant ability to perform. To validate this phenotypic classification and gain deeper insights into the genetic structure of the mutant lines, molecular characterization using an efficient marker system, such as Single Nucleotide Polymorphism (SNP), would be highly beneficial. This approach would provide a more comprehensive understanding of the genetic diversity and facilitate the selection of superior genotypes for future breeding efforts.

## Heritability of key traits and estimated genetic gains

In the present study, broad-sense heritability ( $H^2$ ) estimates for key traits ranged from 1.8% (time to first flowering) to 96.4% (seed length), indicating a spectrum of genetic control from low to high. Seed length and grain yield demonstrated both high heritability and high genetic advance over the mean (GAM), suggesting strong genetic control and significant potential for selection gains. These findings aligned with that of Akohoue et al. [9] and Akohoué et al. [16], who reported high heritability for most quantitative traits in Kersting's groundnut, including grain yield, days to flowering, and seed weight, reinforcing the predominance of genetic effects over environmental influences. However, the low heritability observed for traits such as time to first flowering (1.8%) and chlorophyll content (7.5%) suggests that their improvement may require indirect selection or advanced breeding methods, such as genomic selection. The high GAM for seed length (103.7%) and grain yield (85.2%) underscores their suitability for direct selection, as these traits are likely to respond positively to breeding efforts. Overall, these results emphasize the importance of prioritizing high-heritability traits with substantial GAM, such as seed length and grain yield, in breeding programs to accelerate the development of improved varieties. The strength and the significance of the correlations between traits can also be exploited to conduct correlative batch selections.

## **Implication for future research on the crop**

The findings of this study set the stage for breeding strategies aimed at improving Kersting's groundnut. The identification of genotypes combining high-yielding and early-maturing constitute an important stride towards tailoring elite varieties in the species. The wide variation in heritability and genetic gain estimates suggests that traits with high heritability can be directly selected for improvement, while those with moderate to low heritability may require recurrent selection or genomic approaches. The mismatch between the seed coat color of high-yielding mutants and farmer preferences highlights the importance of integrating consumer traits into breeding programs to ensure adoption. Additionally, the dominance of mutant lines among the top-performing genotypes pinpoints the potential of induced mutation breeding in expanding genetic diversity. By leveraging the genetic variability induced by EMS mutagenesis, breeders can develop high-yielding, resilient varieties that significantly enhance the productivity and sustainability of the crop. Future research should focus on assessing the nutritional content of these elite lines in addition to gauging their capability withstand both biotic and abiotic stresses threatening species (e.g., phoma [35]). Molecular characterization using SNP markers can be tapped in to refine genetic relationships and confirm the observed phenotypic diversity. Genome-wide association studies (GWAS) would also be important to identify key loci controlling not only agronomic traits but also other farmers desired traits such as resistance/tolerance to pests and diseases, and abiotic stresses [26], ultimately accelerating genetic gains in Kersting's groundnut improvement. Moreover, evaluating the stability of these high-yielding mutants across diverse environments and elucidating the genetic mechanisms underlying their superior performance would pave the way for their adoption by farmers and integration into cropping systems.

## **Conclusion**

This study illustrated the potential of induced mutation to broaden the genetic basis of *Macrotyloma geocarpum*, enabling a threefold increase in the yield, the most desired breeding traits in the crop. Beyond the cluster 3 identified as the one encompassing promising genotypes, we specifically targeted lines including ML619, ML1026, ML522, ML1021 and ML1022 as key asset to accelerate strides towards obtaining an elite variety combining high yielding, stress tolerance and nutrient contents. The recent publication of the species reference genome should equally help mining genes supporting the expression of these key end-users desired traits in enabling the carrying out of robust genome wide association studies for these target traits.

## Abbreviations

|      |                                 |
|------|---------------------------------|
| CV   | Coefficient of variation        |
| EMS  | Ethyl methanesulfonate          |
| GAM  | Genetic advance over mean       |
| GWAS | Genome-wide association studies |
| KG   | Kersting's groundnut            |
| PC   | Principal component             |
| PCA  | Principal component analysis    |
| SNP  | Single Nucleotide Polymorphism  |
| USD  | United State Dollar             |
| XOF  | West African CFA francs         |

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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## Author Contribution

ACOA, HO and EGAD: designed the study; ACOA and SV: performed the experimental work; ACOA and DAT: Analyzed the data; ACOA, DAT and AMS: write the original draft; HO, NVFH, HO and EGAD reviewed the manuscript. All authors have read and approved the manuscript.

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## Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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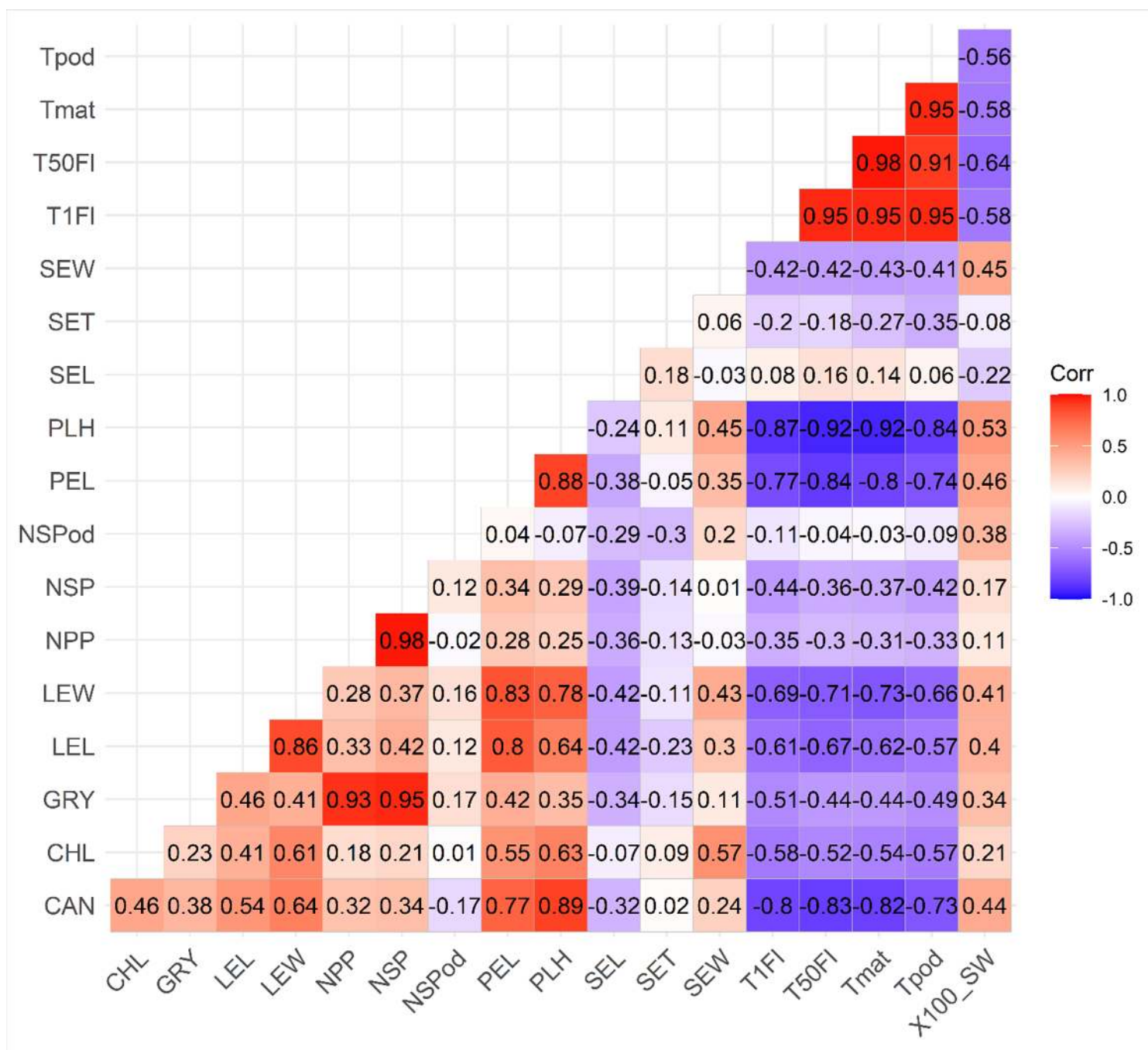
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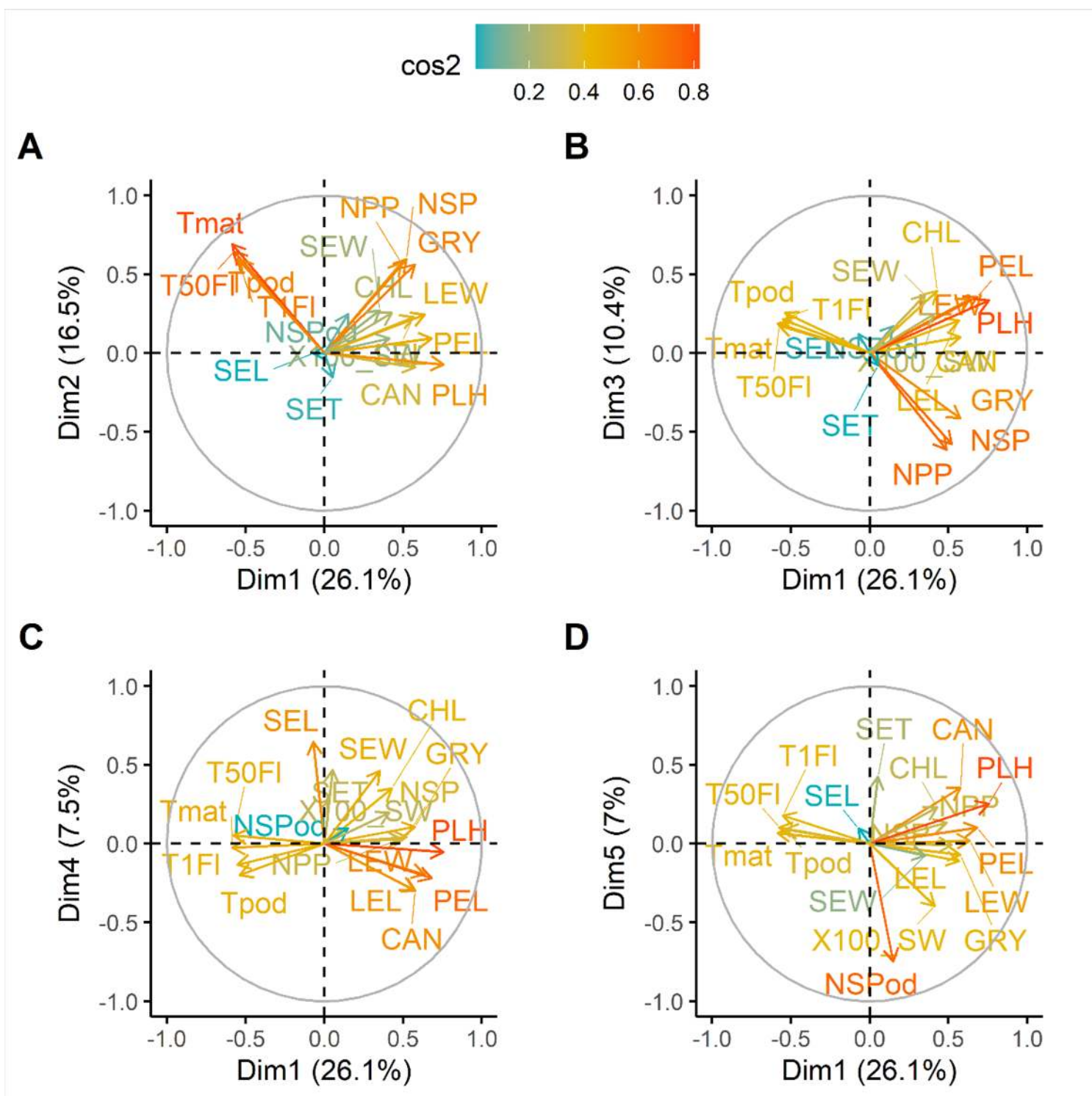
## Figures



**Figure 1**

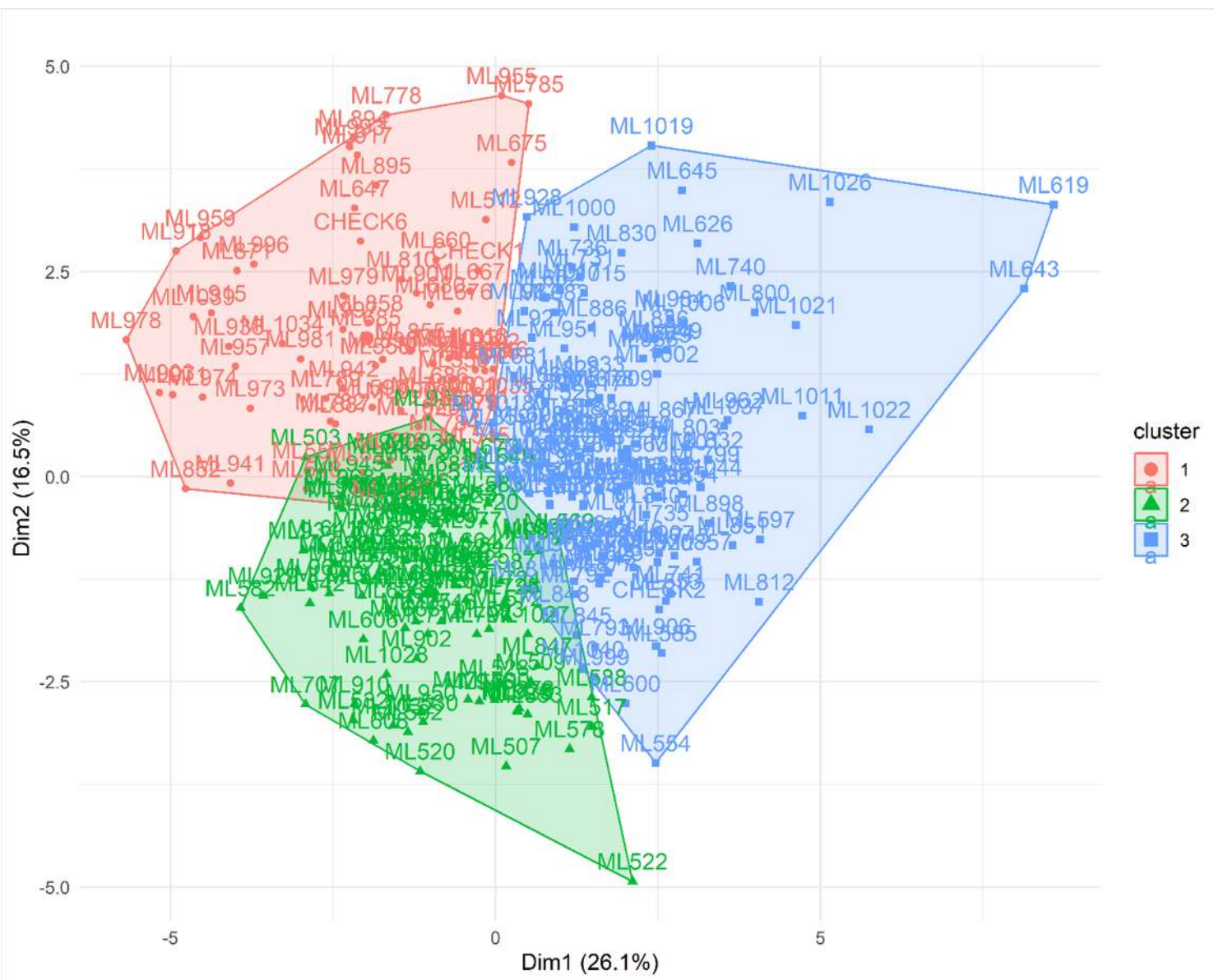
Correlogram depicting the strength of the relationship among the 18 parameters studied.

CHL: Chlorophyl Content; GRY: Seed yield; Leaflet Length; LEW; Leaflet width; NPP: Number of Pod per Plants; NSP: Number of seed per plant; NSPod: Number of Seed per pod; PEL: Petiole length; PLH: Plant length; SEL: Seed Length; SET: Seed thickness; SEW: Seed width; T1FI: Time to first flowering; T50FI: Time to fifty percent flowering; Tmat: Time to maturity; Tpod: Time to podding ; X100\_SW: Hundred seed weight.



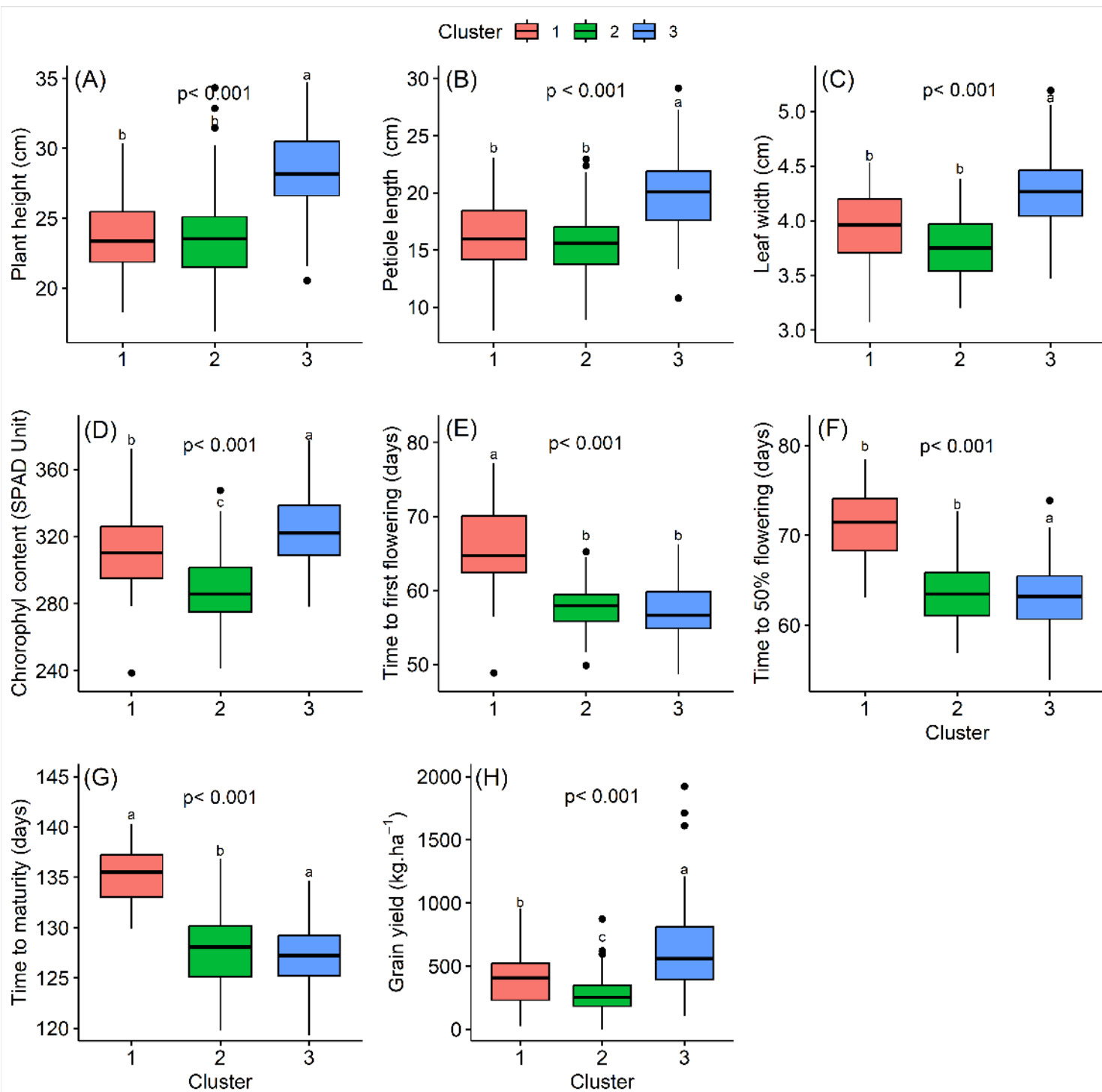
**Figure 2**

Correlation circle describing the positioning of the 18 variables studied on factorial plan defines by dimensions 1-2 (A), 1- 3 (B), 1 – 4 (C) and 1 -5 (D). CAN: Canopy; CHL: Chlorophyl Content; GRY: Seed yield; Leaflet Length; LEW; Leaflet width; NPP: Number of Pod per Plants; NSP: Number of seed per plant; NSPod: Number of Seed per pod; PEL: Petiole length; PLH: Plant length; SEL: Seed Length; SET: Seed thickness; SEW: Seed width; T1F1: Time to first flowering; T50F1: Time to fifty percent flowering; Tmat: Time to maturity; Tpod: Time to podding; X100\_SW: Hundred seed weight.



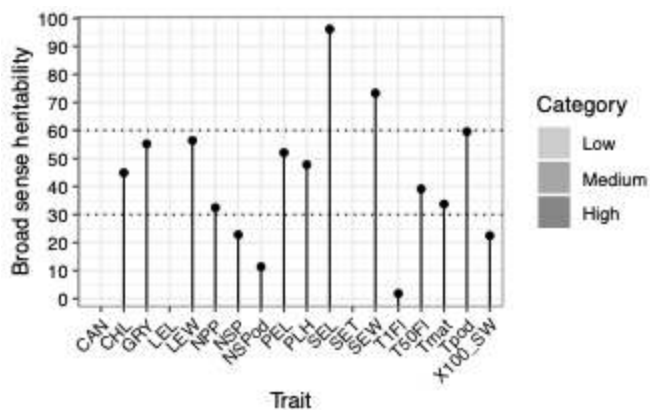
**Figure 3**

Clustering of the 305 genotypes studied into three groups.



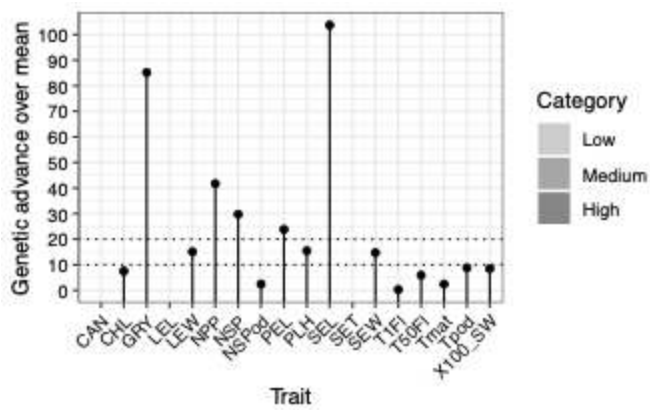
**Figure 4**

Performance comparisons for discriminative traits [(A): Plant height; (B): Petiole length, (C): Leaf width, (D): Chlorophyll content, (E): time to first flowering, (F): Time to 50% flowering, (G): Time to maturity, (H): Grain yield)] among phenotypic groups.



**Figure 5**

Heritability estimates for 15 traits in *Macrotyloma geocarpum* mutants.



**Figure 6**

Variation of the genetic advance over mean across phenotypic traits in traits.