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EFFECTS OF SODIUM AZIDE ON PHENOLOGY, GRAIN FILLING PERIOD AND MATURITY OF COWPEA (*Vigna unguiculata* (L.) Walp.)

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Abstract
Breeding approaches such as mutation breeding can be used for the continuous improvement of cowpea. This study was designed to determine the effects of sodium azide on the phenology, grain filling period and maturity of cowpea. The varieties FUAMPEA 1 and 2, and SAMPEA 17 were treated with sodium azide at concentrations of 0.01, 0.02, 0.03, 0.04 and distilled water as a control. The M_1 was evaluated in a field experimental laid out in a Randomized Complete Block design with three replications. Data was collected on emergence %, DFF, D50%F, D100%F, GFP, EGFP, D95%M, Seed yield and analyzed. Results showed that Sodium azide suppressed germination (emergence) of mutants. The suppression increased as dosage increased. Measurement of Skewness and kurtosis of traits in the M_1 population showed that Emergence % and DFF could be controlled by dominant and complementary gene action, while D50%F, D100%F, GFP, EGFP, D95%M could be controlled by dominant and duplicate epistasis. Sodium azide was found to bring about a mutagenic effect in cowpea plants. There was a highly significant difference in the effects of various doses used on the phenology, grain filling period and maturity of cowpea measured. 0.03% dose was the most effective in reducing D50%F and D100%F, while 0.01% showed positive effect on seed yield. 0.04% dose showed reduction effect on all the traits evaluated, giving a positive reduction for DFF, D95%M and GFP. Our results show that 0.01 % dose of NaN_3 should be used in mutation breeding of cowpea for improvement of seed yield while 0.04 % dose should be employed if reduction in flowering time, maturity and grain filling period of cowpea is the objective. Correlation analysis revealed that number of days to 95 % showed positive and significant association with days to first flower (flower initiation and), as well as grain filling period and effective grain filling period. A positive and significant association was also observed among the mutants for grain filling period and Seed yield.

Key words: Cowpea, Sodium azide, Skewness, Kurtosis, Variances

Introduction
Cowpea, (*Vigna unguiculata* (L) Walp.) is an annual herbaceous legume (family *Fabaceae*) grown predominantly in most developing countries including Nigeria and is an important staple crop providing an affordable source of protein (Muranaka *et al.,* 2016). Young leaves and immature pods are eaten as vegetables (Dugje *et al.*, 2009). The grain is rich in protein (25%), carbohydrates, vitamins, and minerals, and this complements the mainly cereal diet in countries that grow cowpea as a major food crop (Krasova-Wade *et al*., 2006). Aside the protein rich dry grains, Dugje *et al.* (2009) reported that the young leaves and immature pods are eaten as vegetables. Cowpea therefore plays an important role in human nutrition, food security, and income generation for both farmers and people trading on it in the region according to African Agricultural Technology Foundation (AATF, 2006).

The concept of early maturity in cowpea is a combination of early flower initiation and short grain filling period (Kauret *et al*., 2009). Grain filling, the time between pod set and physiological maturity has been shown to be positively related to grain yield (Monpara, 2011). Reduced time to flowering, grain filling period and maturity will greatly enhance breeding efforts for extra early and early maturing cowpea varieties. The knowledge of crop flowering stages (Phenology) and their variability can help improve crop yield (Mirjana and Vulic, 2005). Grain filling period is critical period of grain growth from flowering to maturity and its influences on grain dry weight and maturity may assist breeding efforts to increase grain yield and decrease time to maturity (Darroch and Baker 2007).

Early maturing cowpea varieties are considered climate smart cultivars (Mortimore *et al*., 1997) an important adaptation to climate variability. According to Armah *et al*. (2010), climate variability has made the onset and termination of rains unpredictable in various agroecological zones resulting in a shift to the cultivation of early maturing varieties by farmers. In Sub Saharan Africa, cowpea suffers considerable damage due to frequent terminal drought (because of climate change) especially during the pod filling stage (Agbicodo, 2009; Armah, 2010). Singh (1986) reported that early maturing varieties escape terminal drought and hence increases production and productivity.

Mutation breeding can be described as the purposeful application of mutations in plant breeding (Pathirana, 2011). Induced mutations have great potentials and serve as a viable approach in genetic improvement of crops (Mehandjiev *et al*., 2001). Useful changes in genes produce raw material (variability) for genetic improvement (Adamu *et al*., 2004). Mutations are induced by chemical mutagens and ionizing radiations. Sodium azide (NaN3) is a chemical found to be a powerful chemical mutagen widely used in mutation breeding (Wen and Liang, 2015). The practical value of induced mutations in plant improvement programmes has been well established (Ajibolu *et al*., 2006).

Successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in several crops (Mshembulla *et al*., 2012). This study was undertaken to investigate the potential of Sodium azide to reduce time to flowering and grain filling period in cowpea towards development of early maturing cowpea varieties. The study also aimed to identify the sodium azide dose for inducement of useful mutations in cowpea.

Methodology

The experiment was carried out during the 2020 cropping season at the Teaching and Research Farm of the Joseph Sarwuan Tarka University, Makurdi. The site is located between Latitude 7.410N and Longitude 8.280E, is 97m above sea level and has well drain sandy loam soil. (www.google earth.com/ viewed 2020)

The experimental material consisted of three genotypes of cowpea namely, FUAMPEA 1, FUAMPEA 2, SAMPEA 14 and Sodium azide. These varieties were obtained from the gene bank of the Molecular Biology Laboratory of the Joseph Saawuan Tarka University Makurdi, while the chemical mutagen (Sodium azide) of analytical grade andwas obtained from a commercial chemical store.

Seed Treatment and Bioassay

700 g of clean and healthy-looking seeds were selected for each of the three varieties of cowpea and soaked in distilled water for 3 hours after a floatation test to determine seed viability. The seeds were drained and air dried on paper towels for 1 hour 30 minutes. A mutagenic solution of sodium azide was prepared according to the following doses: 0.01 %, 0.02 %, 0.03 % and 0.04 %.

The air-dried seeds were then soaked in the prepared mutagenic solutions of sodium azide according to their respective doses and a control (distilled water) for duration of 3 hours. The solution was drained thereafter, and the seeds thoroughly washed under running tap water for 30 minutes. They were then immediately taken to the field for planting while ten seeds per treatment were immediately placed in fresh and labelled petri dishes containing moistened cotton wool for germination test. The petri dishes were placed in an aerated well-lit area of the laboratory and observed for germination. The number of germinated seeds was recorded daily, and at the end of the experiment, the mortality was estimated and recorded.

Field Experiment

The experiment was 3x5 factorial arrangement (3 varieties and 5 mutagen doses = 15 treatment combinations) laid out in a Randomized Complete Block Design (RCBD) replicated three (3) times. Each replication consisted of 15 plots for the 15 treatment combinations and each plot consisted of 4 ridges, each 3 m long. Observations were made from the net plot (2 middle ridges)

Two seeds were sown to a depth of 2-3 cm per hill at an intra-row spacing of 30 cm, on each ridge. After planting, a pre-emergence herbicide (Pendamethaline) was applied to subdue weeds until crop establishment. The herbicide was applied at a dilution of 150 mls per 20 L knapsack sprayer. Thereafter, manual weeding was done at 21 days after sowing and subsequently as was needed to keep a weed clean field.

Nitrogen, Phosphorus and Pottasium were applied in the form of NPK 15:15:15 fertilizer at the rate of 100kg/ha. This is the equivalent of 0.075kg (75g) per plot, applied by side placement as a single dose at one week after planting.7 days after sowing.

Pendamethaline applied at a dilution of 100mls per 20 liters knapsack sprayer from flowering till pod maturity to control insect pests. Imicote also applied at the rate of 30mls per 20-liter knapsack sprayer to control aphids on the field.

Table1. Description of the three Cowpea Varieties used in the Study

S/No	Variety	Description	Pod maturity	Origin
1	FUAMPEA 1 (UAM09-1055-6)	Early maturing and high yielding variety	60-62 days	Federal University of Agriculture Makurdi (Now Joseph Sarwuan Tarka University Makurdi-JOSTUM)
2	SAMPEA 14 (IT99K-573-1-)	Early maturing and high yielding variety	68 days	International Institute for Tropical Agriculture Ibadan
3	FUAMPEA 2 (UAM09-1051-1) 80 DAS JOSTUM	Medium Maturing and high yielding variety	75 days	Federal University of Agriculture Makurdi (Now Joseph Sarwuan Tarka University Makurdi-JOSTUM)

Source: Omoigui *et al*., 2016

Experimental Design and treatment combination

The experiment was a 3x5 factorial arrangement (3 varieties and 5 mutagen doses) laid out in a Randomized Complete Block Design (RCBD) replicated three (3) times. Each replication had 15 plots according to the number of treatment combinations. Each plot was comprised of 4 ridges each 3 m long. Observations were made from the net plot (two middle rows)

Observations were recorded on five randomly selected plants for each plot on the following traits: Emergence percentage, Days to first flowering, Days to 50% flowering. Days to 100% flowering, Days to 95% maturity, Grain filling period, Effective grain filling period, Grain yield (Kg/ha)

Statistical Analysis

Descriptive statistics for measured characteristics was calculated using Minitab version 17 (2013). The genetic control of measured traits was determined by skewness and kurtosis values following the interpretations of Pooni *et al.*, (1977) and Robson (1956) as described by Wibowo and Armaniar (2018), and presented in Table 2.

Analysis of variance (ANOVA) was performed using GenStat version 17 and implemented using G.L.M procedure. Statistically significant means were subjected to a post-hoc test in GenStat version 17 using Duncan Multiple Range Test. (DMRT).

Correlation analysis was done using the CORREL function in Excel in office 365, and significance of correlation coefficients was determined using the approach outlined by Zach (2020) on Statology.org (https://www.statology.org/p-value-correlation-excel/).

Table 2. Gene Action based on the Graph form.

Normality test	Graph form	Gene action/number of genes
Skewness = 0	Normal distribution (Symmetric)	Additive gene action
Skewness < 0	Abnormal distribution (Asymmetric). Negatively skewed	Additive gene action with the effect of duplicate epistasis
Skewness > 0	Abnormal distribution (Asymmetric). Positively skewed	Additive gene action with the effect of complementary epistasis
Kurtosis = 3	Mesokurtic	
Kurtosis < 3	Platykurtic	Many genes (Polygenic). Quantitati
Kurtosis > 3	Leptokurtic	Few genes. Qualitative

Adapted from Dasriani *et al.* (2020)

Results and Discussion

Distribution of measured traits and genetic control in the M_1 generation

Figure 1 is the graphs for the nature of distribution for emergence percentage in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a positive skewness (0.23157) and Platykurtic kurtosis (-1.42808).

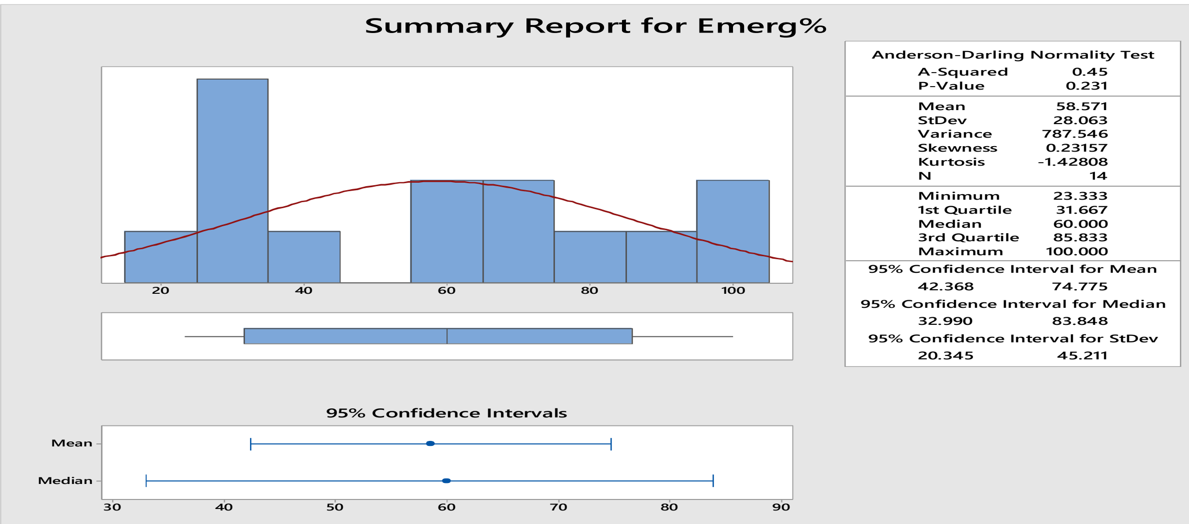

Figure 1: Distribution and Summary Statistics for Emergence Percentage in the First Mutant Population

Figure 2 is the graph for the nature of distribution for days to first flowering in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a positive skewness (0.46217) and Platykurtic (-1.30650).

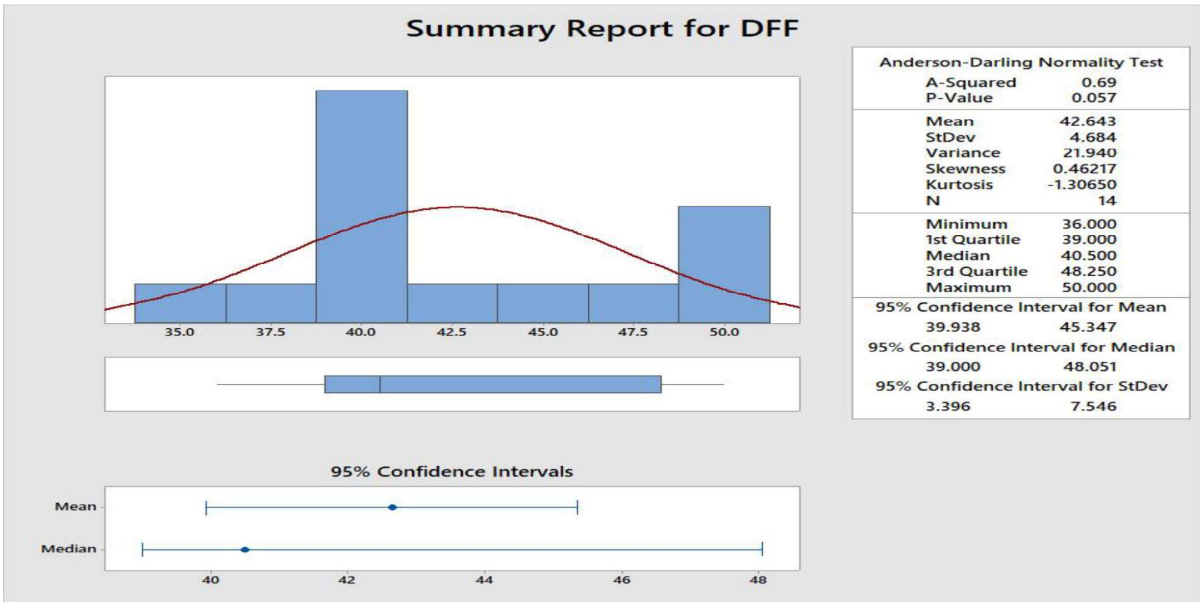

Figure 2: Distribution and Summary Statistics for Days to First Flowering in the First Mutant Population

Figure 3 is the graph for the nature of distribution for days to 50 percent flowering in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.55735) and Platykurtic (0.65867).

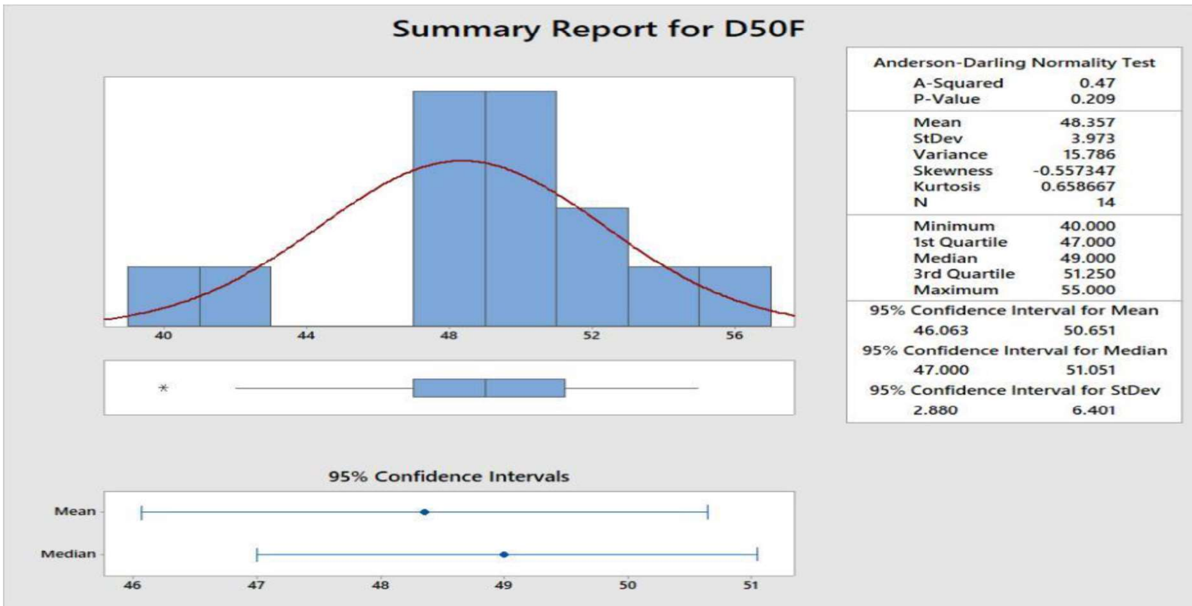

Figure 3: Distribution and Summary Statistics for Days To 50% Flowering in the First Mutant Population

Figure 4 is the graph for the nature of distribution for days to 100 percent flowering in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.62435) and Platykurtic (0.79470).

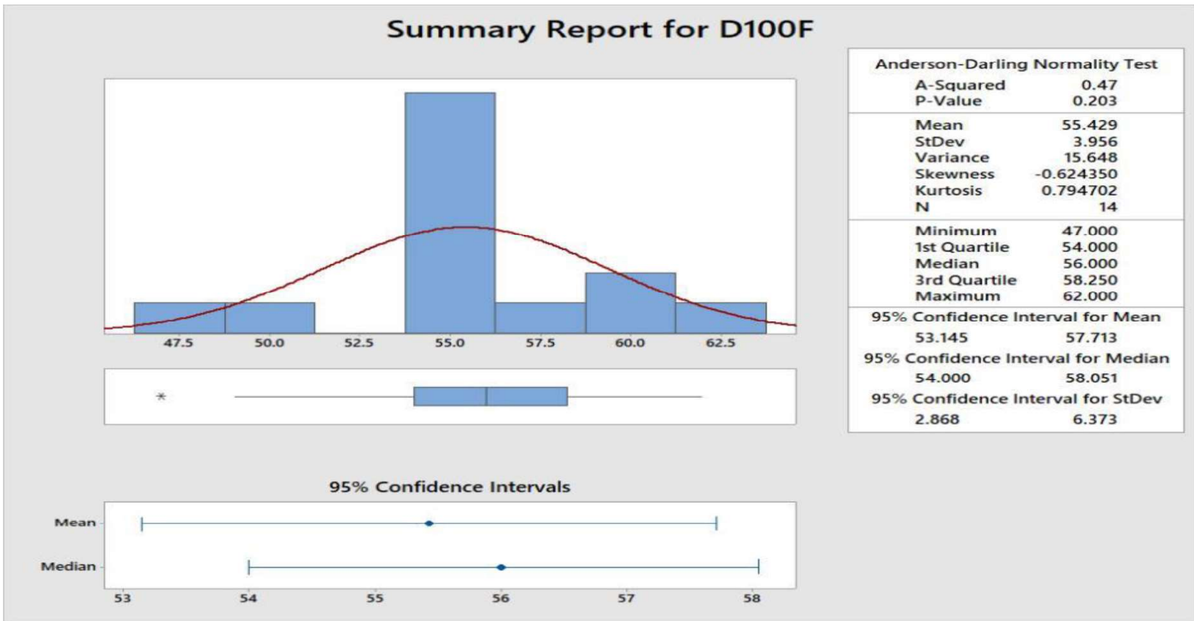

Figure 4: Distribution and Summary Statistics for Days To 100% Flowering in the First Mutant Population

Figure 5 is the graph for the nature of distribution for days to 95 percent maturity in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.62435) and Platykurtic (0.79470).

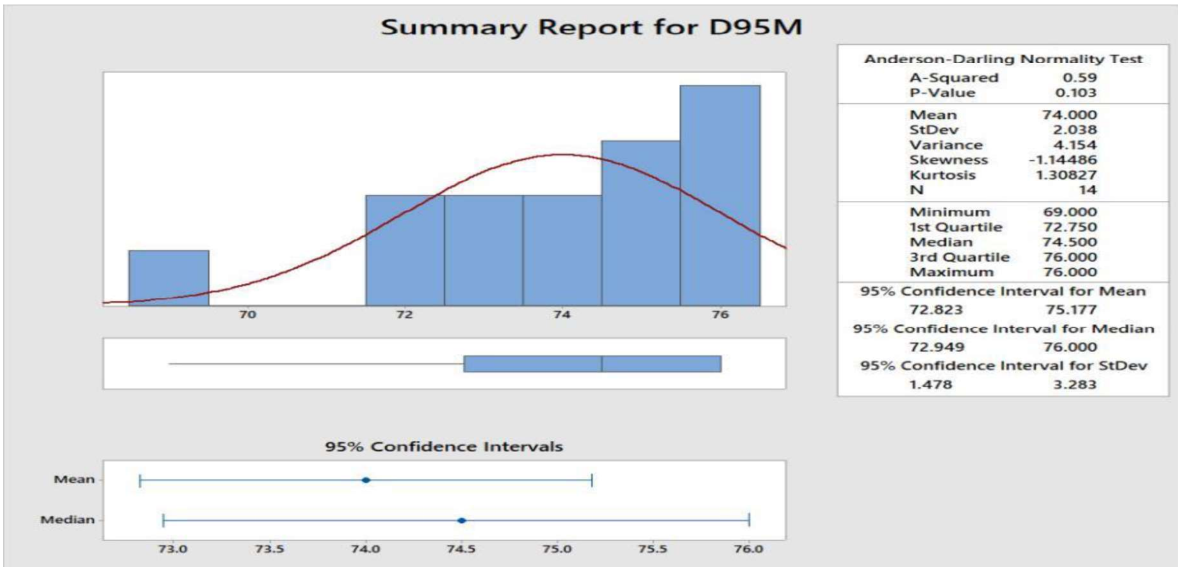

Figure 5: Distribution and Summary Statistics for Days to 95 % Maturity in the First Mutant Population

Figure 6 is the graph for the nature of distribution for grain filling period in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.67941) and Platykurtic (-0.29642).

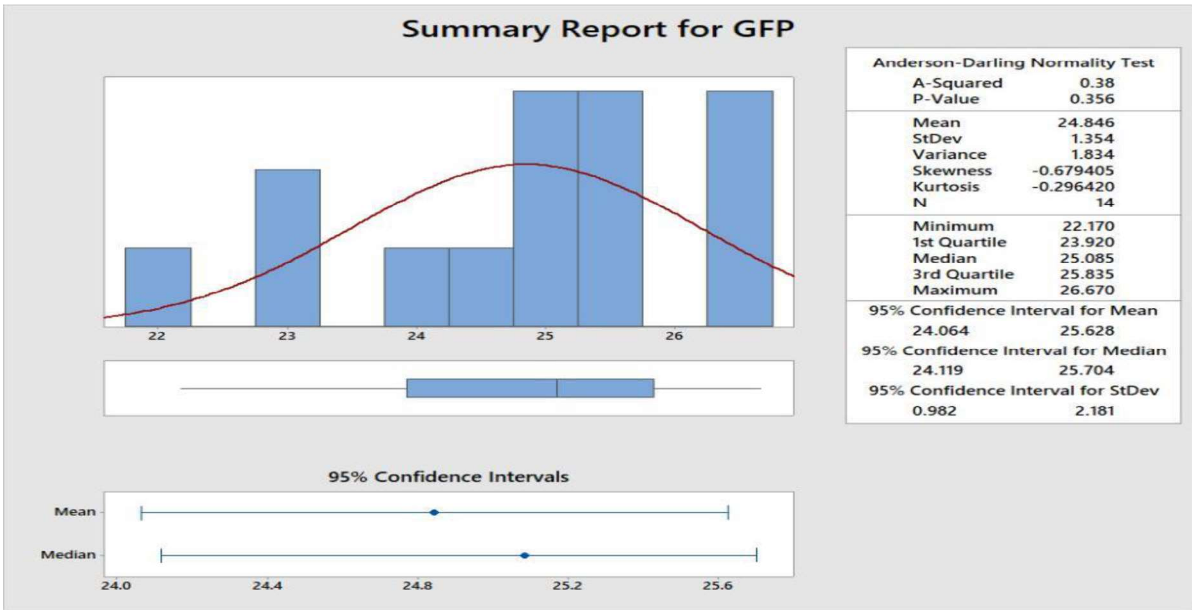

Figure 6: Distribution and Summary Statistics for Grain Filling Period in the First Mutant Population

Figure 7 is the graph for the nature of distribution for effective grain filling period in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.404425) and Platykurtic (-0.966727).

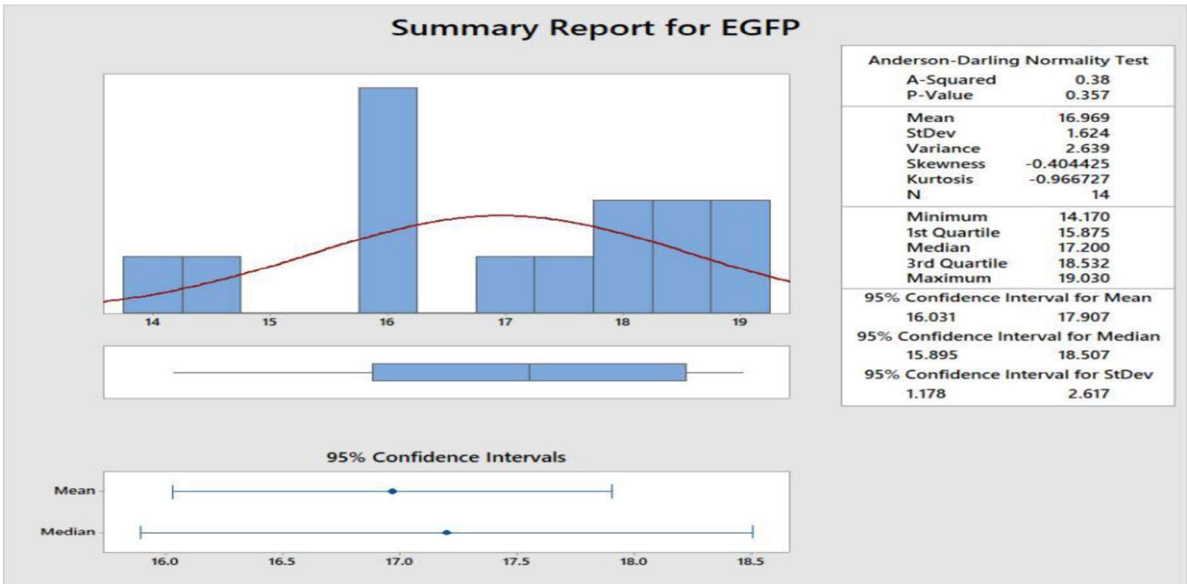

Figure 7: Distribution and Summary Statistics for Effective Grain Filling Period in the First Mutant Population

Figure 8 is the graph for the nature of distribution for Grain yield (Kg/ha) in the population and summary statistics for the trait. The graph shows an asymmetric (abnormal) distribution, with a negative skewness (-0.34761) and Platykurtic (-0.90499).

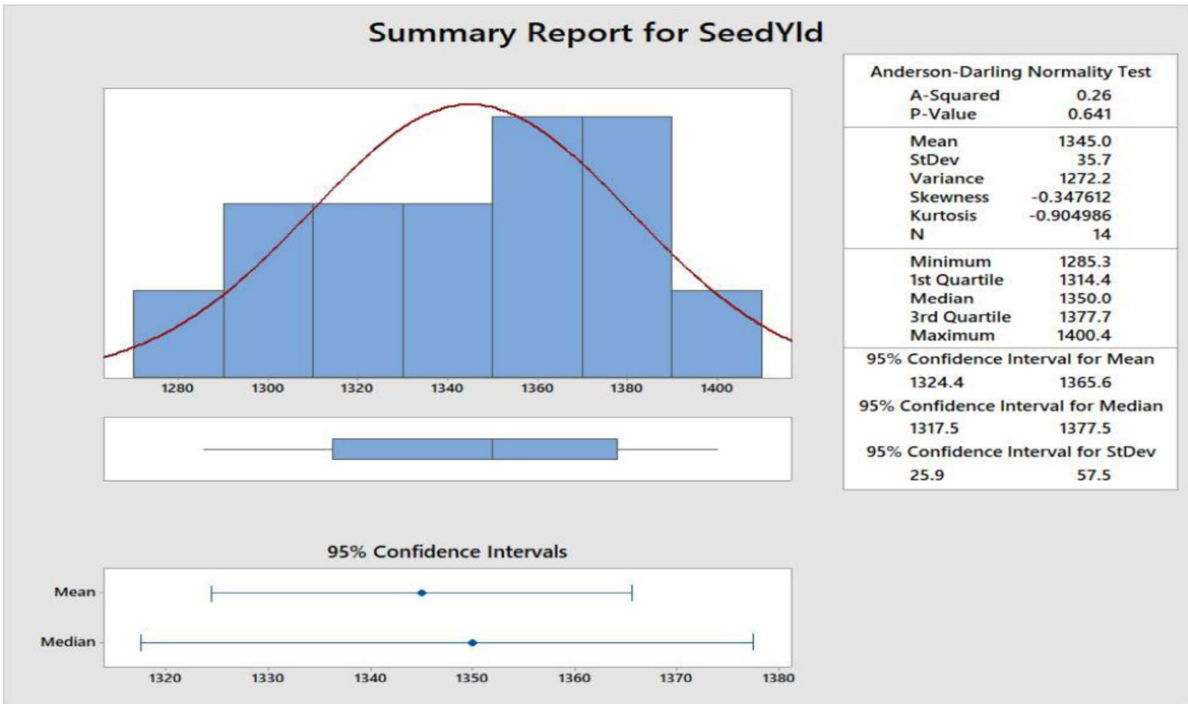

Figure 8: Distribution and Summary Statistics for Grain Yield (Kg/Ha) Period in the First Mutant Population

Estimation of skewness (symmetry) of the distribution of a measured trait in a given population is an important method for determining the genetic control of both quantitative and qualitative traits (Neelima *et al.*, 2020).

The nature of distribution of emergence % and days to first flowering in the mutant population in this study, indicate that they could be controlled by dominant and complementary gene action, while the nature of distribution of days to 50 % flowering, days to 100 % flowering, grain filling period, effective grain filling period, days to 95 % maturity indicate that they could be controlled by dominant and duplicate epistasis (Neelima *et al.*, 2020). The positive skewness for days to first flowering and negative skewness for days to 95 % maturity indicate that sodium azide induced both early and late maturity in the first mutant generation of cowpea. Thus, direct selection for earliness could be effective as reported by Owusu *et al.*, (2020).

In agreement with the findings of Neelima *et al.* (2020), platykurtic distribution was observed for all measured traits of the mutant population are controlled by polygenes, indicating a quantitative genetic variation. This shows the mutagen did not change the quantitative nature of these traits (Shu *et al.,* 2012), but only influenced their expression either positively or negatively. According to Maghuly *et al.* (2018), genetic variability induced mutagenesis offers an opportunity to improve a single character without altering the entire genetic constitution. In addition, the asymmetrical distribution of the mutant individuals in the population for all the observed traits in this study further emphasize that they are under polygenic control (Owusu *et al.*, 2020).

Effects Soduim Azide Treatments on Measured Traits in the First Mutant Generation Emergence percentage (Emerg%)

The character emergence percentage showed a highly significant variation ($p<0.05$) among the varieties and the chemical concentration, however there was no significant effect of variety x concentration interaction on the traits (Table 3). UAM09-1055-6 showed the highest emergence percentage (56.33%), this difference was highly significant different at $p< 0.05$ from the other varieties (Table 4). The least emergence percentage was recorded for IT99K-573-1-1 (42.22 %), it however did not vary statistically from UAM09-1051-1 (45.22%) but significantly varied from the UAM09-1055-6 (Table 4).

Among the chemical concentration studied, the control (0.00%) showed the highest emergence percentage (92.22 %) which was statistically different from the other concentrations (Table 5). The least emergence percentage was observed for 0.04% of NaN_3 (21.11%), it was however not statistically different from 0.03% of NaN_3 (26.30%).

Days to first Flowering (DFF)

The trait days to first flowering showed no significant difference ($p<0.05$) among the varieties, concentration concentrations, and the variety x concentration interaction (Table 3). Among the varieties UAM09-1051-1 and UAM09-1055-6 showed the highest days to first flowering which was 42.43% respectively (Table 4). Among the chemical concentrations, the control showed the highest days to first flowering (44.11%), and this was significantly different from 0.03% dose of NaN_3 (42.56%).

Days to Fifty Percent Flowering (D50F)

The trait days to fifty percent flowering showed a highly significant ($p<0.05$) variation among the varieties and the concentration, while no significant effect was observed for the variety x concentration interaction (Table 3). Among the varieties, IT99k-573-1-1 showed the highest days to fifty percent flowering (50.20) which was statistically different from the other varieties. The least days to fifty percent flowering was shown in UAM09-1055-6 (48.33) (Table 4). Among the chemical concentration studied 0.02% dose of NaN_3 induced the highest days to fifty percent flowering which was (52.00) and this was statistically different from the other concentration and the control. While the least days to fifty percent flowering was shown by the control (46.89) and was significantly different from all the other treatments (Table 5).

Days to Hundred Percent Flowering (D100F)

The trait days to hundred percent flowering showed no significant ($p<0.05$) difference among the varieties, it however showed highly significant variation among the chemical concentrations and no significant difference for the variety x concentration interaction (Table 3).

Among the varieties used in this study IT99k-573-1-1 showed the highest days to hundred percent flowering (56.97), it however did not statistically vary from UAM09-1051-1 (55.50) and UAM09-1055-6 (55.43) (Table 4).

Among the chemical concentration 0.03% dose of NaN_3 induced the highest days to hundred percent flowing (58.78) which was not statistically different from 0.02 % dose of NaN_3 (57.44) but significantly varied from the other treatments. The least days to hundred percent flowing was induced by 0.01% dose of NaN_3 (53.56), it was however not statistically different from control (53.89) (Table 5).

Grain Filling Period (GFP)

The trait grain filling period showed a highly significant ($p<0.05$) variation among the varieties and the concentration, while no significant effect was observed in the interaction (Table 3).

Among the varieties, UAM09-1055-1 showed the highest grain filling period (24.77), and this was statistically ($p< 0.05$) different from UAM09-1055-6 and IT99k-573-1-1. The least grain filling period was observed in IT99K-573-1-1 (23.27) (Table 4).

Among the chemical concentration used, 0.02% dose of NaN_3 induced the highest grain filling period (25.94). The least grain filling period was induced by 0.04% dose of NaN_3 (24.34) which statistically ($p< 0.05$) different from the other treatments.

Effective Grain Filling Period (EGFP)

The effective grain filling period trait showed a highly significant ($p< 0.05$) variation among the varieties and the chemical concentration, it however showed no significant difference for the variety x concentration interaction (Table 3). Among the varieties used in this study, UAM09-1051-1 gave the highest effective grain filling period (20.61), this statistically different ($p<0.05$) from UAM09-1055-6 and UAM09-1055-1-1. The least effective grain filling period was recorded in IT99K-573-1-1 (15.51) (Table 4).

Among the chemical concentrations, the control gave the highest effective grain filling period (19.03), which was statistically different from the other concentration. The least effective grain filling period was induced by 0.02% dose of NaN_3 (16.54), it was however not statistically different from 0.04% dose of NaN_3 (16.64) (Table 5)

Days to Ninety Five Percent Maturity (D95M)

The trait days to ninety five percent maturity (D95M) showed a highly significant ($p<0.05$) variation among the varieties and the chemical concentration, while no significant effect was observed for the interaction.

Among the varieties studied, UAM09-1051-1 showed the highest days to ninety five percent maturity (74.77) which was statistically different from UAM09-1055-6 and IT99k-573-1-1 (64.47 and 74.00) (Table 4).

Among the concentrations the control gave the highest days to ninety five percent maturity (72.33) which was statistically different from the other concentrations. The least days to ninety five percent maturity was induced by 0.01% dose of NaN_3 (70.00) (Table 5).

Seed Yield Kg/ha

The trait seed yield showed no significant ($p<0.05$) variation among the varieties, and the concentration while there was no significant effect for the interaction (Table 3). Among the varieties used in this study, UAM09-1055-6 showed the highest seed yield (1342kg/ha) which was statistically different from the other varieties. The least amount of seed yield was observed from UAM09-1051-1 (1331kg/ha).

Among the chemical concentration studied, 0.01% dose of sodium azide (NaN_3) produce the highest seed yield (1384kg/ha) which was statistically different from the other concentrations and the control. The least seed yield was produced by 0.04% dose of NaN_3 (1290kg/ha) which varied statistically from the concentrations.

The annual average production of cowpea is low compared to other grain legumes, primarily due to narrow genetic variability and the low yielding potential of existing genotypes (Raina *et al.,* 2020). The consistent use of conventional breeding approaches has reduced genetic variability, which is the main prerequisite for crop improvement programs (Holme *et al*., 2019). Therefore, breeding techniques such as induced mutagenesis are required to accomplish the goals of increased genetic variability.

It is evident that sodium azide contributed to the genetic variation in the mutant population in this study. High variability recorded for emergence percentage and Grain yield indicated a significant impact of sodium azide on these traits. The significant effect of sodium azide on measured traits of cowpea in this study, has also been reported by Eze and Dambo (2015), where they stated that all traits and nutritional composition were significantly affected by sodium azide except seedling height, height at maturity, days to fifty percent flowering and plant leaf at maturity. The effect on the phenology, maturity and grain filling period traits among the sodium azide induced mutants of cowpea evaluated, shows the potential of mutation breeding to improve these traits in cowpea. However, the results of this study revealed lower and moderate doses of sodium azide employed individually induced desirable mutations in quantitative traits, especially for phenology. Raina *et al.* (2020) have reports that mutagenic effectiveness and efficiency were maximum at lower and intermediate doses with sodium azide. Higher doses reduced grain filling period but supressed germination. This observation was in agreement with Aminu *et al.* (2017) were there observed that that sodium azide can lead to a decrease in emergence percentage, reduced seedling root, length and decrease in stem girth as the concentration increases.

These results therefore suggest that improvement of traits by induced mutagenesis should be done by using specific doses to target individual traits for desired results.

At interaction level the result indicated no significant effect among all the traits evaluated. This indicates that the mutagen doses produced their effect irrespective of cowpea variety. However, UAM09-1051-1 responded more effectively to sodium azide than the other cowpea varieties, which also suggests that mutagenesis may not be a one type of mutagen fits all the varieties of a specie scenario.

The doses of sodium azide had differential effect on measured traits. 0.01% dose of NaN_3 showed effectiveness in inducing desirable mutation in seed yield, as well as other measured traits. On the other hand, 0.04% dose of NaN_3 induced decrease in almost all the measured traits. This decrease was desirable for time to flowering, maturity and grain filling period. The failure of the highest concentration of the sodium azide treatment to produce desirable effects in some traits is like the report of Eze and Dambo (2015). Similar result concerning effect of sodium azide has been reported in African Yam bean (Tayo *et al*., 2021). Such reduction might be due to toxicity of the mutagen on physiological parameters. It is equally possible that some mutant genes or positive alleles were recessive and thus failed to be expressed in the M_1 generation.

Table 3. Mean squares from Analysis of Variance for effect of Sodium azide on measured traits

Sources of Variation	Emerg%	DFF	D50F	D100F	GFP	EGFP	D95M	SeedYld
Variety	828.95**	1.62ns	13.27ns	11.27ns	48.25**	104.27**	493.91**	441.5ns
Concentration	7376.91**	17.89ns	36.19**	45.58**	5.05**	10.70**	7.77**	13297.4**
Var x Concentration	64.60ns	1.87ns	3.66ns	1.88ns	0.34ns	0.78ns	1.32ns	333.8ns
Error	29.59	9.16	5.50	8.24	0.33	0.91	0.81	71.3
CV (%)	11.3	7.2	4.8	5.10	4.75	5.4	1.3	1.0

**** = Highly significant at P ≤0.05. Emerg% =Emergence percentage, DFF =Days to first flowering, D50F =Days to 50 percent flowering, D100F =Days to hundred percent flowering, GFP =Grain filling period, EGFP =Effective grain filling period, D95M =Days to 95 percent maturity, SeedYId =Seed yield Kg/ha.**

Table 4. Main effect of Variety on Phenology, Maturity and Grain filling period of cowpea

Variety	Emerg%	DFF	D50F	D100F	GFP	EGFP	D95M	SeedYld
IT99K-573-1-1	42.22a	41.90a	50.20b	56.97a	23.37a	15.51a	64.47a	1336ab
UAM 09-1051-1	45.22a	42.43a	49.0 ab	55.50a	26.93c	20.61c	74.77c	1331a
UAM 09-1055-6	56.33b	42.43a	48.33a	55.43a	24.77b	16.90b	74.00b	1342b
Mean	47.93	42.28	49.20	55.97	25.02	17.68	71.08	1336.2
SE Mean	1.986	1.11	0.86	1.04	0.21	0.35	0.33	4.78

Means within a column with similar letter are not significantly different at P≤0.05. Emerg% =Emergence percentage, DFF =Days to first flowering, D50F =Days to 50 percent flowering, D100F =Days to hundred percent flowering, GFP =Grain filling period, EGFP =Effective grain filling period, D95M =Days to 95 percent maturity, SeedYId =Seed yield Kg/ha

Table 5. Main effect of Sodium azide on Phenology, Maturity and Grain filling period of cowpea

Concentration	Emerg%	DFF	D50F	D100F	GFP	EGFP	D95M	SeedYld
0.04	**21.11a**	**40.17a**	**49.67bc**	**56.17ab**	**24.34a**	**16.64a**	**70.50a**	**1290a**
0.03	**26.30a**	**42.56ab**	**52.00c**	**58.78b**	**24.22a**	**18.43bc**	**71.67bc**	**1306b**
0.02	**42.22b**	**42.11ab**	**49.78bc**	**57.44b**	**25.94c**	**16.54a**	**70.89ab**	**1342c**
0.01	**57.78c**	**42.44ab**	**47.67ab**	**53.56a**	**25.05b**	**17.73b**	**70.00a**	**1384e**
0.00 (Control)	**92.22d**	**44.11b**	**46.89a**	**53.89a**	**25.56bc**	**19.03c**	**72.33c**	**1359d**
Mean	**47.93**	**42.28**	**49.20**	**55.97**	**25.02**	**17.68**	**71.08**	**1336.2**
SE Mean	**2.5**	**1.43**	**1.11**	**1.35**	**0.27**	**0.45**	**0.42**	**6.17**

Means within a column with similar letter are not significantly different at P≤0.05. Emerg% =Emergence percentage, DFF =Days to first flowering, D50F =Days to 50 percent flowering, D100F =Days to hundred percent flowering, GFP =Grain filling period, EGFP =Effective grain filling period, D95M =Days to 95 percent maturity, SeedYId =Seed yield Kg/ha

Correlation between Measured traits of the First Mutant Generation

The association between phenological traits, grain filling period, maturity and seed yield of the first mutant generation of cowpea is shown in Table 6. Grain filling period had a positive and highly significant association with days to first flower, while it was negatively and significantly associated with days to 50 % flowering. Effective grain filling period had a positive and significant association with days to first flower and a positive and highly significant association with grain filling period. It had a negative and highly significant association with days to 100 % flowering. Days to 95 % maturity had a positive and highly significant association with days to first flower, grain filling period and effective grain filling period. It had a negative and highly significant association with days to 50 % flowering and days to 100 % flowering. Seed yield had a positive and highly significant association with grain filling period and effective grain filling period.

The concept of early maturity in cowpea is a combination of early flower initiation and short grain filling period (Kauret *et al*., 2009). Early maturing cowpea varieties are considered climate smart cultivars (Mortimore *et al.,* 1997) an important adaptation to climate variability. According to Armah *et al*. (2010), climate variability has made the onset and termination of rains unpredictable in various agroecological zones resulting in a shift to the cultivation of early maturing varieties by farmers.

In the present study number of days to 95 % showed positive and significant association with days to first flower (flower initiation and), as well as grain filling period and effective grain filling period. This suggest that the longer it takes the mutants to flower and fill grain, the longer it will take them to mature. Days to first flowering and grain filling period can thus be used together as indirect selection criteria for maturity as previously reported by Kauret *et al*. (2009).

A positive and significant association was also observed among the mutants for grain filling period and Seed yield, indicating that the longer the mutant genotypes take to fill grain the higher the seed yield will be. A negative and significant association between days to maturity and seed yield recorded in this study suggest that early maturing genotypes could also be high yielding. These findings will suggest that mutants could combine long grain filling period, early maturity and high seed yield.

Table 6. Association between Phenological Traits, Grain Filling Period, Maturity and Seed Yield of the First Mutant Generation of Cowpea

TRAIT	DFF	D50F	D100F	GFP	EGFP	D95M	SeedYld Kg/ha
DFF	1						
D50F	-0.93541**	1					
D100F	-0.99919**	0.948875ns	1				
GFP	0.798357**	-0.53386*	-0.77347ns	1			
EGFP	0.710607*	-0.41592ns	-0.68171**	0.991009**	1		
D95M	0.997746**	-0.90957**	-0.99424**	0.836966**	0.756219**	1	
SeedYld Kg/ha	0.052414ns	-0.40213ns	-0.09256ns	0.55951*	0.665376**	-0.01472ns	1

*** =Significant at P≤0.05, ** =Highly significant at P≤0.05, Emerg% =Emergence percentage, DFF =Days to first flowering, D50F =Days to 50 percent flowering, D100F =Days to hundred percent flowering, GFP =Grain filling period, EGFP =Effective grain filling period, D95M =Days to 95 percent maturity, SeedYId =Seed yield Kg/ha**

Conclusion

Emergence % and days to first flowering in the mutant population in this study could be controlled by dominant and complementary gene action, while days to 50 % flowering, days to 100 % flowering, grain filling period, effective grain filling period, days to 95 % maturity could be controlled by dominant and duplicate epistasis.

Sodium azide is a mutagen and has been one of the most effective mutagens in crop plant and in this study, it has been found to bring about a mutagenic effect in cowpea plants. There was a highly significant difference in the effects of various concentration of sodium azide on the phenology and grain filling period traits of cowpea measured. 0.03% dose played the most effective role in days to fifty percent flowering and days to hundred percent flowing while 0.01% dose showed effectiveness in seed yield. 0.04% dose showed reduction effect on all the traits evaluated, giving a positive reduction for days to first flowering, maturity and grain filling period.

Our results show that 0.01 % dose of NaN_3 should be used in mutation breeding of cowpea for improvement of seed yield while 0.04 % dose should be employed if reduction in flowering time, maturity and grain filling period of cowpea is the objective.

Correlation analysis revealed that number of days to 95 % showed positive and significant association with days to first flower (flower initiation and), as well as grain filling period and effective grain filling period. A positive and significant association was also observed among the mutants for grain filling period and Seed yield.

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