Development of High Yielding Cowpea [Vigna unguiculata (L.) Walp.] Lines with Improved Quality Seeds through Mutation and Pedigree Selection Methods

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Abstract: Cowpea [Vigna unguiculata (L.) Walp.] is a major legume crop and an important source of protein in Africa. The Kafr El-Sheikh University has a long history of cowpea breeding and improvement in Egypt. Two superior lines with high seed yield and quality were selected through mutation breeding and released to farmers as new varieties under the names Kafr El Sheikh-1 and Kaha-1. Crosses were made between these two varieties to further improve cowpea to meet farmers’ demand. Using the pedigree selection method, 13 new superior F10 lines were selected and evaluated over 2 years for seed yield and related traits, earliness, and protein content under low (16 plants/m2) and high (24 plants/m2) plant densities. The results showed that plants grown in narrower space produced significantly higher seed yield per unit area than the plants grown in wider space. All developed lines produced significantly higher seed yield than the two parental lines in the 2018 trial and Kaha-1 in the 2019 trial. Line number 6 proved to be the best genotype for earliness (73.5–73.9 days after sowing), seed yield (573–647 g/m2), and crude protein content (22.7–24.3%) in both trials. In addition, line 4 with bushy determinate growth habit and high seed quality was recently released as a new variety (Sakha-1). Several other cowpea lines have clear potential for release as new high-yielding varieties with early maturity and high seed quality for farmers in Egypt. Seeds of selected lines are available from Kafrelsheikh University. This shows that mutation breeding and pedigree selection methods are among the most promising breeding methods for cowpea improvement.

Keywords: field evaluation; plant density; protein content; legume crop; seed yield; Vigna

1. Introduction

Cowpea [Vigna unguiculata (L.) Walp.] is an important legume crop in developing countries, with 80% of production occurring in the dry savannas of tropical West and Central Africa [1]. Cowpea can be used in the form of dry seeds, fodder, green pod, green manure, and cover crops. It is mainly cultivated in Africa, including Egypt, for its dry seeds and/or green pods before maturity as a vegetable. Cultivated cowpea is a valuable source of protein, micronutrients, and vitamins [2]. This crop can grow in a wide variety of soils ranging from well-drained heavy clay to sand, as well as dry environmental conditions [3,4]. Cowpea can be used as intercrop with cereals, which improves soil fertility [5]. In addition, Rhizobia bacteria in root nodules of legumes fix atmospheric nitrogen at about 240 kg per
ha and improve soil fertility and structure [6]. However, increasing abiotic stress such as drought, heat, and salinity, as well as high pressure from diseases and insect pests under climate change, reduce the yield and quality of existing legume varieties [7].

Besides abiotic and biotic stresses, plant density also plays an important role in determining the full seed yield potential of grain legume cultivars. The use of appropriate plant density is critical for optimum seed yield in legumes such as cowpea. There are several factors that affect plant density, such as soil moisture, available nutrients, crop management, and genotype [8]. In addition, the optimum plant density varies from region to region [9–13]. Cowpea varieties with different plant morphologies require different optimal densities to maximize seed yield and crop quality [14–16].

To date, genetic improvement of cowpea has lagged behind vegetable crops in Egypt. Production has been limited for many years to a few varieties that are relatively prostrate, late-maturing, and produce few seeds. A better understanding of legume diversity and the use of crop wild relatives in breeding can broaden the current narrow genetic basis in legumes including cowpea [17,18]. Mutation breeding, purification of already established varieties, and development of superior cowpea lines through pedigree selection are among the most promising breeding methods for cowpea. Resistance to cowpea aphids was reported in mutant lines (ICV 11 and ICV 12), which were expressed through antibiosis and antixenosis [19]. These mutant lines were obtained from ICV 1 seeds irradiated with 20 k rad of gamma rays. The pedigree system of breeding is the most common method used by cowpea breeders, which has been successful in developing cowpea cultivars with new combinations of characteristics and resistance to diseases [12,20].

Through mutation breeding, two improved M$_5$ lines were selected and released by Kafrelsheikh University and made available to farmers as the two best local cowpea varieties [Kafr El-Sheikh-1 and Kaha-1] at Kafr El-Sheikh region and then distributed throughout Egypt within a few years. Both varieties have bushy determinate growth habit and higher seed yield and quality than the old local variety Cream-7 [12]. In the present study, crosses were made between Kafr El-Sheikh-1 and Kaha-1 to increase genetic variability and select new cowpea lines with superior agronomic performance by pedigree selection. In addition, these lines were evaluated for earliness, seed yield traits, and crude protein to develop superior lines better adapted to climate change conditions. Several lines displayed early-maturing and higher seed yield than the parental varieties. These have clear potential for release as new varieties for farmers in Egypt. High yielding cowpea lines will be used to make crosses with wild *Vigna* species for development of pre-breeding lines for adaption to climate change.

2. Materials and Methods

2.1. Development of New Cowpea Lines

Cowpea seeds of variety Cream-7 were originally exposed to three doses of gamma radiation (10, 20, and 30 kr). Seeds were sown in April 1991 and 250 plants from each treatment were selected for M$_2$ seed production. M$_2$ plants were evaluated, and 25 mutant lines were selected and characterized by vigorous, bushy, determinate growth habit, high pod and seed yield, early maturity, and larger seeds than Cream-7. The mutant lines were evaluated during M$_3$ and M$_4$ generations, and the lines KFS-107 and KFS-61 were selected with the above superior traits. These lines were released in 1998 as new varieties for cultivation in Egypt under the names Kafr El-Sheikh-1 and Kaha-1 [12]. Crosses were made between Kaf El-Sheikh-1 and Kaha-1 as a female parent and Kaha-1 as male parent to produce F$_1$ seeds in the summer of 2006. About 80 F$_1$ plants were self-pollinated to produce F$_2$ seeds and then 2000 F$_2$ plants were evaluated for the desired traits at three stages of plant growth: flowering, pod setting, and seed maturity. Selections for desired traits were repeated in subsequent generations from F$_3$ until F$_{10}$. A total of 13 promising improved lines were selected in the F$_{10}$ generation in 2016 and designated as lines 1, 2, 3, 4, 5, 6, 8, 9, 23, 28, 35, 53, and 56. Overall, 13 promising cowpea lines were developed by pedigree selection.
according to Poehlman and Sleper [21] (Table 1) and evaluated under field conditions over two seasons.

### Table 1. Breeding history of 13 cowpea lines derived from the crosses between the two local varieties Kafr El-Sheikh-1 and Kaha-1.

| Year | Generation | Breeding and Selection Activities |
|------|------------|----------------------------------|
| 2006 | F₁         | Crosses between Kafr El-Sheikh-1 (female) and Kaha-1 (male) |
| 2007 | 80 F₁ plants | Grow 80 F₁ plants to generate F₂ seeds |
| 2008 | 2000 F₂ plants | Select best individual F₂ plants |
| 2009 | 200 F₃ plants | Grow F₃ plants, select the best rows within selected families, and select best plants within selected rows |
| 2010 | 110 F₄ families | Grow F₄ lines, select the best rows within selected families, and select best plants within selected rows |
| 2011 | 64 F₅ families | Grow F₅ lines, select best rows within selected families, and select best plants within selected rows |
| 2012 | 35 F₆ lines | Grow F₆ lines, select the best families, and harvest best rows in bulk |
| 2013 | 20 F₇ lines | Grow F₇ lines, select the best families, and harvest best rows in bulk |
| 2014 | 13 F₈ lines | Testing F₈ lines and harvest best seed yield plots from one replication in bulk |
| 2015 | 13 F₉ lines | Testing F₉ lines and bulk best seed yield plots from one replication to initiate pure seed development |
| 2016 | 13 F₁₀ lines | Testing F₁₀ lines and bulk best seed yield plots from one replication to increase pure seed |

### 2.2. Evaluation of Promising Cowpea Lines under Field Conditions

Selected lines along with their parents, Kafr El-Sheikh-1 and Kaha-1, were evaluated in field trials during the 2018 and 2019 summer seasons for early maturity, seed yield, and crude protein content under two plant densities, i.e., 16 plants/m² (80 cm between ridges and 30 cm within rows) and 24 plants/m² (80 cm between ridges and 20 cm within rows) at the experimental farm, Faculty of Agriculture, Kafrelsheikh University (latitude 31°6'42" N, longitude 30°56'45" E). Mechanical and chemical analyses of the experimental soil are presented in Table 2.

### Table 2. Mechanical and chemical analysis of the experimental field soil (0–30 cm depth) in the 2018 and 2019 trials.

| Variable       | Trial |
|----------------|-------|
|                | 2018  | 2019  |
| Mechanical analysis |       |       |
| Sand%          | 10.00 | 9.20  |
| Silt%          | 32.40 | 31.90 |
| Clay%          | 57.60 | 58.90 |
| Textural class | Clay  | Clay  |
| Chemical analysis |      |       |
| pH             | 7.80  | 8.00  |
| EC dsm-1       | 3.31  | 3.30  |
| Organic matter%| 1.93  | 1.80  |
| Available N ppm| 17.60 | 19.00 |
| Available P ppm| 7.60  | 7.70  |
| Available K ppm| 280.00| 265.00|

The evaluation trials were set up using a split-plot design with four replications. The two plant densities were arranged in the main plot and the cowpea genotypes (13 lines and two parental varieties) in sub-plots. Seeds were sown in hills using two plant densities of 16 plants/m² and 24 plants/m². The experimental plot (8 m²) contained one ridge of
10 m long and 0.8 m wide, and each hill had two plants. The recommended agricultural practices of the Egyptian Ministry of Agriculture were applied in both field trials in 2018 and 2019. Days to maturity was recorded as the number of days from sowing to maturity of 90% of the pods in each plot. Seed yield was determined by harvesting all pods in a 1 square meter area. The pods were threshed and weighed to obtain grain yield. Seed yield and its components were determined as seed yield g/m², number of pods/plant, number of seeds/pod, seed index (weight of 100-seeds), and seed protein content. Nitrogen in the digested dry seeds was determined by the micro-Kjeldahl method according to Carter [22], and nitrogen in the dry seeds was multiplied by a factor of 6.25 to calculate the crude protein content.

2.3. Statistical Analyses

Statistical procedures were performed using SAS (version 9.1; SAS Institute, Cary, NC, USA). Days to maturity, seed yield, and crude protein data were subjected to analysis of variance (ANOVA) followed by a Duncan’s Multiple Range test at the 0.05 probability level [23].

3. Results

The ANOVA showed significant differences among genotypes for all the traits in the 2018 and 2019 summer seasons (Figures 1 and 2). Significant differences were observed between plant density for seed yield and crude protein, and non-significant differences for days to maturity and number of pods per plant in both seasons (Table 3). Interaction between genotypes and plant density was significant for traits except for the number of pods per plant and number of seeds per pod in 2018, and days to maturity in the 2019 summer season (Table 3). This shows the differential response of genotypes for most of the traits under different plant densities.

![Figure 1. Overview of plant morphology of 13 selected F10 lines compared to the parental lines Kaha-1 and Kafr El-Sheikh-1.](image-url)
4. Discussion

Cowpea is a major legume crop, which is rich in protein, carbohydrates, vitamins, and minerals, and complements the mainly cereal diet in countries that grow cowpea as a major food crop [1,2]. The old cowpea varieties in Egypt such as Cream-7 were late-maturing (>150 days) and produced low seed yield (<95.23 g per m²). Here, we improved old cowpea varieties and developed new high-yielding early-maturing cowpea lines that are preferred by farmers.

The results showed that five new cowpea lines were early and extra-early maturing lines (65 to 77 days), which reached 90% of pod maturity earlier than their parental varieties (Kafr El-Sheikh-1 and Kaha-1). This may be attributed to the fact that pod maturity in cowpea is more genetically controlled and less influenced by the environment [24]. Cowpea cultivars are generally classified into three categories in terms of pod maturity according to Ehlers and Hall [25]: extra-early (<60 days from sowing to pod maturity), early (61–75 days from sowing to pod maturity), and late maturity (>80 days from sowing to pod maturity). Harvesting in Egypt is done by handpicking the pods, and old varieties (indeterminate and prostrate) require three harvesting times or more. Our study developed new early-maturing cowpea lines that can be harvested in one time to save time and efforts.

In the present study, plants grown at high plant densities produced higher seed yields per unit area than plants grown at low plant densities. This may be attributed to the accommodation of a greater number of plants producing seeds per unit area under high plant density planting. Previous studies have also reported similar results wherein total yield was increased under high plant density [26–28]. On the contrary, increasing plant density reduced the number of pods per plant, and seed index, thereby reducing the seed yield per plant. This could be due to the increased competition among plants for available soil nutrients, moisture, light, and carbon dioxide [27], resulting in reduced translocation and accumulation of photosynthates from source to sink and thereby affecting overall plant growth and hence reduced seed yield per plant. Farmers in Egypt prefer to use high plant density (24 plants per meter square) for planting erect cowpea varieties. Newly developed 13 cowpea lines with bushy, determinate growth habit may require.

Table 3. Analysis of variance for different traits among 13 advanced breeding lines along with their parents evaluated under two plant densities (16 and 24 plants/m²) for earliness, seed yield components, and crude protein during 2018 and 2019 summer season at Karf El-Sheikh region.

| Source of Variation | Degree of Freedom | Mean Squares |
|---------------------|-------------------|--------------|
|                     |                   | Days to Maturity | Seed Yield (Kg/m²) | No. of Pods/Plant | No. of Seeds/Pod | Seed Index (g/100 Seeds) | Crude Protein (%) |
| Replication         | 3                 | 0.5           | 29,614.47       | 4.092           | 0.519            | 5.811                   | 0.005                 |
| Plant density (A)   | 1                 | 0.033ns       | 830,003.30 **   | 0.044ns         | 5.002ns          | 24.3 **                 | 0.442 **              |
| Error               | 3                 | 1.92          | 8210.097       | 5.353           | 2.292            | 0.122                   | 0.001                 |
| Genotype (B)        | 14                | 179.8 **      | 150,088.57 **  | 171.636 **      | 12.807 **        | 61.508 **               | 8.158 **              |
| A × B               | 14                | 10.64 **      | 6973.59 **     | 3.523ns         | 1.452ns          | 1.889 **                | 0.692 **              |
| Error               | 84                | 0.735         | 1333.29        | 3.969           | 1.001            | 0.104                   | 0.001                 |
| Grand Mean          |                   | 78.15         | 498.87         | 19.361          | 8.711            | 20.783                  | 23.248                |
| CV (%)              |                   | 1.1           | 7.32           | 10.29           | 11.54            | 1.55                    | 0.16                  |

2018 Summer Season

| Replication         | 3                 | 1.156         | 222,256         | 3.146           | 0.063            | 26                      | 0.018                 |
| Plant density (A)   | 1                 | 2.133ns       | 1,256,244.033 **| 3.008ns        | 4.219 **         | 6.533ns                 | 9.163 **              |
| Error               | 3                 | 0.756         | 1874.522       | 2.521           | 0.173            | 16.044                  | 0.011                 |
| Genotype (B)        | 14                | 187.401 **    | 30,972.705 **  | 32.068 **       | 11.629 **        | 85.873 **               | 5.709 **              |
| A × B               | 14                | 0.437ns       | 5079.676 *     | 2.182 *         | 0.207 *          | 4.301 **                | 2.694 **              |
| Error               | 84                | 0.527         | 2827.841       | 1.281           | 0.09             | 2.004                   | 0.011                 |
| Grand Mean          |                   | 80.633        | 483.383        | 18.2            | 8.232            | 19.433                  | 23.104                |
| CV (%)              |                   | 0.9           | 11             | 6.22            | 3.65             | 7.29                    | 0.46                  |

* Significant at p ≤ 0.05; ** Significant at p ≤ 0.01; ns = non-significant; CV = Coefficient of variation.
Table 4. Effect of plant density and genotypes on seed yield traits and crude protein of cowpea lines evaluated in 2018 and 2019 trials.

| Factors | Days to Maturity (2018) | Seed Yield (g/m²) | No. of Pods/Plant | No. of Seeds/Pod | Seed Index (g/100 Seeds) | Crude Protein % | Days to Maturity (2019) | Seed Yield (g/m²) | No. of Pods/Plant | No. of Seeds/Pod | Seed Index (g/100 Seeds) | Crude Protein % |
|---------|------------------------|-------------------|-------------------|------------------|------------------------|----------------|------------------------|-------------------|-------------------|------------------|------------------------|----------------|
|         |                        |                   |                   |                  |                        |                |                        |                   |                   |                  |                        |                |
| Plant density |                      |                   |                   |                  |                        |                |                        |                   |                   |                  |                        |                |
| 16 plants/m² | 78.2                   | 415.7 b             | 19.4              | 8.9              | 21.3 a                 | 23.3 a         | 80.5                   | 381.1 b             | 18.4              | 8.4 a             | 22.3 b             |                      |                |
| 24 plants/m² | 78.1                   | 582.0 a             | 19.3              | 8.5              | 20.4 b                 | 23.2 b         | 80.8                   | 585.7 a             | 18.0              | 8.0 b             | 22.8 a             |                      |                |
| F-test     | ns                     | ns                 | ns                | ns               | ns                     | ns             | ns                     | ns                | ns                | ns               | ns                     | ns             |

** indicate significance at \( p < 0.0001 \), respectively. Means with the same letter within the same column are not significantly different at \( p \leq 0.05 \) according to Duncan's multiple range test; each value represents the mean of four replicates.

As observed in ANOVA, the results presented in Tables 4 and 5 also showed that plant density had no significant effect on earliness in terms of days from sowing to 90% pod maturity in both 2018 and 2019 seasons. Conversely, genotypes had highly significant effects on earliness in both seasons. The parental lines reached 90% pod maturity at 81.0–81.8 days from sowing (Kafr El-Sheikh-1) and 78.9–79.5 days (Kaha-1) in the 2018 and 2019 seasons, respectively. Five new cowpea lines reached 90% of pod maturity earlier (65.4 to 77.3) than their parental lines. Regarding the interactions between plant density and genotypes for earliness, there were significant differences in the 2018 season (Tables 3 and 5). The results showed that line number 28 was the earliest in low and high plant densities, reaching maturity at 67.1 and 65.4 days in the 2018 and 2019 seasons, respectively (Table 5).

Significant differences were observed between plant densities for seed yield (Table 3). Plants grown at high plant densities produced significantly higher seed yields per unit area (24/m²) than plants grown at low plant densities (16/m²) (Tables 4 and 5). On the contrary, increasing plant density reduced the number of pods per plant, and seed index, thereby reducing the seed yield per plant.

The differences between genotypes were highly significant for seed yield and its components in both seasons (Tables 3 and 4). There were also significant differences between genotypes at low and high plant densities for seed yield in the 2018 trial (Table 5). Interestingly, all 13 cowpea lines produced significantly higher seed yield than parents in the 2018 trial, whereas only two lines (6 and 28) exhibited significantly higher seed yield than parents in the 2019 trial (Table 4). Line 6 had the highest seed yield in 2018 season and line 28 in 2019 season at low and high plant densities in both trials (Table 5). In addition, lines 6, 28, and 56 had the highest number of seeds per pod in both trials. Bold seeds (seed index) were found in lines 6 and 53 in the 2018 trial and lines 1, 3, 5, 9, and 56 in the 2019 trial.
Table 5. Effect of interaction between plant density and genotypes on seed yield traits and crude protein in cowpea lines evaluated with their parents in 2018 and 2019 trials.

| Factors (Genotype x Plant Density) | 2018 Trial | 2019 trial |
|-------------------------------------|------------|------------|
| Plant No. of Seed Index Crude Protein Days to Maturity Yield (g/m²) No. of Plants No. of Seeds Yield (g/100 seeds) Maturity No. of Seeds/ Pod No. of Pods Plant Seed |
| Days to Maturity | (g/m²) | No. of Pods/ Plant | No. of Seeds/ Pods | Seed Index (g/100 seeds) | Crude Protein (%) | Days to Maturity | (g/m²) | No. of Seeds/ Pod | No. of Pods/ Plant | Seed Index (g/100 seeds) | Crude Protein (%) |
| 1 x 16 | 80.5 b–d | 348.0 k | 13.8 | 6.3 | 24.3 b | 22.7 q | 79.5 | 286.0 kl | 6.3 op | 21.6 a | 23.0 ab | 21.1 n |
| 2 x 16 | 70.0 i | 472.8 gh | 18.5 | 8.8 | 18.5 k | 23.3 m | 70.8 | 380.0 h–j | 9.0 de | 14.9 k | 19.5 ef | 21.7 m |
| 3 x 16 | 80.3 b–d | 464.8 gh | 18.7 | 7.8 | 22.5 d | 23.1 n | 79.8 | 335.0 j–l | 10.1 a | 18.9 f–i | 23.5 ab | 22.1 k |
| 4 x 16 | 83.3 e | 390.8 jk | 20.2 | 8.5 | 20.8 gh | 24.5 c | 81.8 | 419.0 fj | 8.6 e–g | 16.6 g–k | 19.5 ef | 23.4 de |
| 5 x 16 | 80.8 bc | 400.8 i–k | 18.9 | 7.6 | 25.8 e | 23.6 i | 79.8 | 389.0 g–f | 7.8 i–m | 13.1 j | 21.5 b–e | 22.5 hi |
| 6 x 16 | 73.3 j | 534.0 ef | 25.5 | 11.0 | 21.3 fg | 24.1 f | 73.5 | 480.0 ef | 10.0 a | 16.3 h–k | 20.0 d–f | 22.5 hi |
| 7 x 16 | 80.8 bc | 439.2 h–j | 22.7 | 8.3 | 16.3 j | 23.6 i | 79.3 | 361.0 h–k | 8.9 def | 16.5 p | 23.3 i | 22.6 k |
| 8 x 16 | 80.8 bc | 451.6 g–j | 19.6 | 9.3 | 23.3 c | 23.5 j | 79.8 | 446.0 f–i | 8.0 i–l | 18.9 b–f | 21.5 b–e | 22.4 ij |
| 9 x 16 | 84.3 a | 468.8 gh | 20.5 | 8.9 | 22.5 d | 23.3 m | 82.5 | 357.0 k–l | 7.3 n | 19.3 b–c | 20.0 d–f | 23.0 fg |
| 10 x 16 | 78.6 j | 478.8 f–h | 23.1 | 10.4 | 21.3 fg | 20.2 u | 65.3 | 430.0 f–i | 10.0 a | 17.3 f–j | 21.5 b–e | 21.6 m |
| 11 x 16 | 80.8 b | 462.5 gh | 21.5 | 9.0 | 20.8 gh | 23.5 j | 80.3 | 384.0 h–j | 6.7 o | 19.4 a–d | 16.5 gh | 20.7 o |
| 12 x 16 | 73.5 g | 512.4 e–g | 16.6 | 8.4 | 17.0 t | 23.8 g | 73.0 | 396.0 f–j | 8.1 g–j | 18.6 c–f | 16.5 gh | 22.3 i–k |
| 13 x 16 | 84.3 a | 468.8 gh | 20.5 | 8.9 | 22.5 d | 23.3 m | 82.5 | 357.0 k–l | 7.3 n | 19.3 b–c | 20.0 d–f | 23.0 fg |
| 14 x 16 | 78.6 j | 478.8 f–h | 23.1 | 10.4 | 21.3 fg | 20.2 u | 65.3 | 430.0 f–i | 10.0 a | 17.3 f–j | 21.5 b–e | 21.6 m |
| 15 x 16 | 78.5 ef | 104.8 m | 8.4 | 7.7 | 18.3 k | 22.4 s | 79.8 | 275.0 l | 8.1 h–k | 21.6 a | 16.5 gh | 22.0 kl |
| 16 x 16 | 80.5 b | 248.1 i | 15.8 | 9.1 | 22.8 d | 23.1 n | 81.8 | 403.0 f–j | 7.7 n | 18.1 d–h | 19.5 ef | 22.8 gh |
| Kaha-1 x 16 | 81.5 b | 487.8 f–h | 15.5 | 6.2 | 24.5 b | 23.5 j | 79.8 | 450.0 h–i | 6.4 o | 19.8 b–e | 24.0 a | 22.5 hi |
| El-Shiekh-1 x 16 | 79.5 c–e | 487.8 f–h | 15.5 | 6.2 | 24.5 b | 23.5 j | 79.8 | 450.0 h–i | 6.4 o | 19.8 b–e | 24.0 a | 22.5 hi |

Crude protein content was significantly affected by plant density, genotypes, and their interaction in both seasons (Tables 3–5). Under low plant density, line 56 had the highest crude protein content in both trials (25% in 2018 and 24.8% in 2019), whereas this line behaved differently under high plant density in 2018 and 2019 trials. Evaluation of cowpea lines is available at: https://ics.hutton.ac.uk/cwr/cowpea (accessed on 17 August 2021), which has allowed us to publish and make the data from this work available online. This has a number of advantages including facilitating unrestricted access of data to researchers, providing them with tools to explore and visualize their data, aligning with other cowpea data and allowing the downloading of data in standard formats. The two datasets that we have available for this work cover 11 different traits across 2018 and 2019 for 15 cowpea accesses and four replications. In total there are 2640 data points. The datasets can be visualized from within Germinate or downloaded using the following links: 2018 https://ics.hutton.ac.uk/cwr/cowpea/#/data/export/trials/18 (accessed on 17 August 2021) and 2019 https://ics.hutton.ac.uk/cwr/cowpea/#/data/export/trials/19 (accessed on 17 August 2021).

4. Discussion

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In the present study, plants grown at high plant densities produced higher seed yields per unit area than plants grown at low plant densities. This may be attributed to the accommodation of a greater number of plants producing seeds per unit area under high plant density planting. Previous studies have also reported similar results wherein total yield was increased under high plant density [26–28]. On the contrary, increasing plant density reduced the number of pods per plant, and seed index, thereby reducing the seed yield per plant. This could be due to the increased competition among plants for available soil nutrients, moisture, light, and carbon dioxide [27], resulting in reduced translocation and accumulation of photosynthates from source to sink and thereby affecting overall plant growth and hence reduced seed yield per plant. Farmers in Egypt prefer to use high plant density (24 plants per meter square) for planting erect cowpea varieties. Newly developed 13 cowpea lines with bushy, determinate growth habit may require lesser inputs of water and fertilizer [29,30] and are best suited for high-density planting as preferred by the farmers in Egypt.

Concerning seed yield, three cowpea lines exhibited significantly higher seed yield than parents in both trials. The components of seed yield in cowpea consist of three main traits, i.e., number of pods/plant, number of seeds/pod, and average seed weight. These components were superior in lines 6, 28, and 53 compared to those in the other lines and original parents. In this context, Metwally et al. [30] indicated that mutation breeding could improve seed yield components of cowpea. In addition, Aliya and Makinde [24] reported that cowpea cultivars differ genetically in the expression of yield components, such as number of pods per plant, number of seeds per pod, seed index, and seed weight.

Differences in seed yield traits among selected cowpea lines and parents in the 2018 and 2019 trials could also be attributed to environmental variations such as temperatures, light intensity, and humidity [12,30]. Interestingly, line 4 was released as new cowpea variety (Sakha-1) to farmers in Egypt in 2020. This variety has a bushy determinate growth habit, is early maturing, and has a high seed yield and protein content. Seeds of Sakha-1 have white hilum color and take a short cooking time, which are preferred traits by consumers.

Metwally et al. [29] indicated that there was no clear relationship between plant density and crude protein content in dry seeds of cowpea. In the present study, crude protein content was significantly affected by plant density, genotypes, and their interaction. Differences in the studies could be due to the use of different cowpea genotypes and/or differences in the growing environments. Nielsen et al. [31] also reported that cowpea cultivars differed in nutrient composition including protein content. Resistance to insect-pests such as cowpea bruchids (Callosobruchus sp.), which cause significant losses in cowpea in Egypt, is still lacking in these lines. In general, the majority of cowpea losses is mostly attributed to biotic stress [32], in particularly insect-pests [33]. Resistance to insect-pests has been detected in wild Vigna species such as V. hirtella, V. minima, V. nepalensis, V. riukiuensis, V. tenuicaulis, V. umbellata, V. reflexopilosa ssp. glabra, and V. trinervia [17]. So far, no pest-resistant varieties have been developed using these wild species because of the linkage drag of undesired traits and crossing barriers [34]. Further research is required for using wild
relatives in cowpea breeding for developing biotic and abiotic resistant/tolerant varieties having better adaptation to climate change.

5. Conclusions

In the present study, 13 new superior lines with high seed yield, early maturity, and improved quality seeds were developed through mutation breeding and pedigree selection methods. Evaluation of these lines under different plant densities over seasons showed that these newly developed lines have higher yield potential than the parental lines and are the best suited for high-density planting in Egypt. Further, most of these lines are more early-maturing than parents, which makes them suitable for short-cropping seasons. It is interesting to note that one of these high-yielding lines, line 4 with high seed protein and bushy determinate growth habit, was recently released as a new variety, Sakha-1, in 2020. Further, line 6 having early maturity, high seed yield, and high crude protein content along with a few other high-yielding lines identified in the present study, has clear potential for release as new high-yielding varieties with early maturity and high seed quality for farmers in Egypt. Efforts will be made to introgress other useful traits such as insect-pest resistance into these lines by using wild species. Seeds of the new cowpea lines are available for seed distribution from breeders at Kafrelsheikh University, and evaluation data of cowpea lines is available through the Germinate platform [35,36] (https://ics.hutton.ac.uk/cwr/cowpea (accessed on 17 August 2021).

Author Contributions: Conceptualization, E.M. and M.R.; methodology, E.M., M.R. and M.S.; validation, E.M. and M.R.; formal analysis, M.S., M.R., S.S., E.M., P.D.S., S.R. and A.M. (Ali Masry); investigation, E.M., M.R. and M.S.; resources, E.M. and M.R.; data curation, E.M., P.D.S., S.R., B.K., A.M. (Ali Masry) and M.R.; writing—original draft preparation, E.M. and M.R.; writing—review and editing, M.R., E.M., B.K., S.S. and A.F.; visualization, M.R.; supervision, E.M., A.M. (Ali Masoud) and M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported in part by the project “Evaluation and propagation of new superior lines from cowpea and garlic for releasing new varieties to farmers in Egypt” which is funded by the Academy of Scientific Research and Technology in Egypt http://www.asrt.sci.eg/ (accessed on 17 August 2021) to release new cowpea varieties in Egypt. This work has also been supported in part by the project “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives” which is supported by the Government of Norway to make the evaluation data of cowpea lines available through the Germinate platform.

Data Availability Statement: The data presented in this study are available from the Germinate platform at: https://ics.hutton.ac.uk/cwr/cowpea (accessed on 17 August 2021) or downloaded using the following links: 2018 https://ics.hutton.ac.uk/cwr/cowpea/#/data/export/trials/18, (accessed on 17 August 2021) and 2019 https://ics.hutton.ac.uk/cwr/cowpea/#/data/export/trials/19, (accessed on 17 August 2021).

Acknowledgments: This work has been supported in part by the project “Evaluation and propagation of new superior lines from cowpea and garlic for releasing new varieties to farmers in Egypt” which is funded by the Academy of Scientific Research and Technology in Egypt http://www.asrt.sci.eg/ (accessed on 17 August 2021), to release new cowpea varieties in Egypt. This work has also been supported in part by the project “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives” which is supported by the Government of Norway to make the evaluation data of cowpea lines available through the Germinate platform. The project is managed by the Global Crop Diversity Trust with the Millennium Seed Bank of the Royal Botanic Gardens, Kew UK and implemented in partnership with national and international genebanks and plant breeding institutes around the world (https://www.cwrdiversity.org/ (accessed on 17 August 2021).

Conflicts of Interest: The authors declare no conflict of interest.
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