Middle East Research Journal of Biological Sciences
ISSN 2789-7710 (Print & ISSN 2958-2091 (Online)
Frequency: Bi-Monthly
DOI: 10.36348/merjbs.2024.v04i01.003

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Genetic Purity Analysis of faba bean (*Vicia faba L.*) Produced in Different Seed Companies of Ethiopia

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Abstract: The best way to boost agricultural productivity and output is using high-quality seed. Genetic purity is tainted during the development of hybrid seeds by selfing events or out-crossing with different types. In order to assess the genetic purity of fababean varieties produced in various Ethiopian seed firms, this study used the agro-morphological and grow-out test method to analyze the genetic purity of the fababean crop. Two distinct seed classes of the fababean crop, Tumsa and Wolki, were gathered from various sources. To evaluate the genetic purity of the seed, 1 kg of each variety was gathered from the highland pulse breeding department of the Holetta Agricultural Research Center, Oromia Seed Enterprise, and Ethiopia Seed Enterprise. The study was carried out over the course of two consecutive, independent years in 2020 and 2021, using a randomized complete block design with three replications. All growth traits of the obtained data showed significant differences at ($p<0.05$) in the analysis of variance results, with the exception of the number of leaflets per leaf and the number of nodes per plant. In general, the current results indicated both seed homogeneity as well as heterogeneity in the agro-morphological and grow-out tests. This suggests that various seed suppliers possess different standards for creating high-quality seed. Therefore, each and every seed producer needs to be made aware of the quality seed production procedures and techniques that they must adhere to. In the same way, additional genetic purity analysis might be carried out within the seed system to guarantee error repair, assurance, and control over seed quality.

Keywords: Agro-morphological, Genetic, Grow-out test, Purity, Quality, Seed.

RESEARCH PAPER

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How to cite this paper:
Elsabet Bayisa *et al* (2024). Genetic Purity Analysis of faba bean (*Vicia faba L.*) Produced in Different Seed Companies of Ethiopia. *Middle East Res J Biological Sci, 4*(1): 17-21.

Article History:
| Submit: 23.12.2023 |
| Accepted: 24.01.2024 |
| Published: 31.01.2024 |

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important legumes for its seed high protein content and nutritional value (Crépon *et al.*, 2010). The crop is widely cultivated both use in human food and animals feed. The world production of faba beans reaches 4.3 Million tons from total cultivated area of 2.55 Million hectares (FAO STAT, 2012). One of the most important limitative factors of the production potential of any crop is represented by the seed quality in which standards of germination percentages, moisture contents of seed, thousand seed weight and seed purity are considered under seed quality. Quality seed is the key for development of agricultural activity. Genetic variation among faba bean genotypes is very important for their efficient utilization in plant breeding schemes and effective conservation. It is estimated that the use of quality seed of a variety having high genetic potential (as for the improved varieties) can increase yield by 20 – 25% (Tekalign, Derera and Sibiya, 2020). Level of genetic contamination are depend severely on the nature of the crop. Cross pollinated crops like faba bean and maize contamination level can be very higher than self-pollinated crops (Cabrera, Collins and Salgado, 2006). In seed production scheme, purity level of produced seed can be vary from crop to crop as well as for different seed classes. During production contamination can be occur because of different reason like large area production (un-manageable field) and lack of skill in seed production techniques. Once the quality of produced seed are contaminated, it may passed from generation to generations. This can be addressed by evaluating genetic purity level of the crop through molecular method by DNA sequencing as well as agro-morphological traits using grow-out test methods. Though morphological and agronomic traits are routinely used to access genetic diversity, they are not enough in numbers to cover the genome, can be affected by environmental factors and developmental stage dependent. However in somehow, to detect the contamination level of limited number of variety, morphological evaluation and agronomic traits can be useful to identify purity level of the seed at different classes. Therefore, this experiment was

conducted for the objective to analyze genetic purity of different fababean variety produced by various seed growers in the country through determining the level of phenotypic off types by grow out test method (GOT).

2. MATERIALS AND METHODS

2.1. Study sites

The field experiments were conducted at Holetta Agricultural Research Centre (HARC) (09º03'N, 38º30'E, and 2390 masl) classified as M2-5(Tepid to cool moist mid highlands) agro ecology. The soil type of HARC experimental fields is Eutric Nitosols, and the annual average precipitation is 933.3 mm with average minimum and maximum temperature of 5.3ºC and 22ºC respectively.

2.2. Method of data collection

Ten individual plants were randomly selected for each genotype per each replication from the central part of the row and were marked before flowering. All morphological data were collected from the 10 marked plants. All growth and seed yield data were recorded carefully at desired stage.

2.3. Data sources

2.3.1. Plant materials

Two of commercial fababean varieties namely Tumsa and Wolki were collected from different seed growers of Ethiopia. For each variety, one (1)kg sample was collected from different sources like from regional seed growers (Oromia seed enterprise), from federal seed growers (Ethiopia seed enterprise) and Holetta Agricultural research centere (pulse crop research team). During sample collection, different seed class (pre-basic and basic) was collected from seed growers and breeder seed from HARC and used as control for the experiment (Table-1).

Table-1: List of experimental materials and Treatment combinations

Treatments	Seed source+ variety + seed class
T1	ETB (Ethiopia seed interprise, tumsa and basic)
T2	ETPB (Ethiopia seed interprise, tumsa andP/ basic
T3	HTBr (breeder seed of tumsa from holetta)
T4	OTB (tumsa basic seed from oromia seed interprise)
T5	OWB (basic seed of wolki from oromia SE)
T6	HWBr (breeder seed of wolki from holetta)
T7	EWB (basic seed of wolki fom Ethio. Seed interprise)
T8	EWPB (p/basic seed of wolki from ESE)

2.4. Experimental design and management

The experimental plots of the sites had uniform slope and thus a randomized complete block design with three replication was used. Two hundred Fifty seeds of each genotype were planted on six rows of 2.4 m long and 0.4 m inter-row spacing. The trials were managed in accordance with the standard practice for the site. This included regular hand weeding to keep field clean of weeds, application of the recommended fertilizer DAP at the rate of 100 kg/ ha with 18 kg/ ha Urea at planting. Planting was done at the onset of the main season rainfall.

4. Data Analysis

4.1. Statistical analysis

The data were first subjected to the individual year analysis (ANOVA), then tested for homogeneity of variance for the sites using Bartlett's homogeneity test (Tekalign, Derera and Sibiya, 2020) (Littell, 1989) in SAS/STATA (SAS Institute, 2012).The homogeneity of the error variance allowed the combined analysis of variance to be performed and the two independent growing season data were analyzed by using R-software of simple randomized completely block design.

5. RESULTS AND DISCUSSION

5.1. Growth Traits

The result from the experiment revealed that there is a variation between the varieties for various growth parameters. However, there is no significance difference ($P< 0.05$) between the treatments for parameters like number of leaflet per leaf and number of node per plant for both years.

A statistical investigation revealed that the kinds of faba beans used had significant effects on plant height, leaf breadth, flower quantity, bloom length, and leaflet length in addition to days to emergency. In comparison to other treatment combinations, the results indicated that the tumsa variety, which was collected from both seed growers (ESE and OSE), took the shortest time to emerge. On the other hand, the wolki variety, which was collected from the Horn of Africa Research Center (HARC), took the longest to emerge. Consequently, basic seed of the tumsa variety obtained from Ethiopian and Oromia seed businesses recorded the longest height, while the wolki variety from ESE registered the lowest height. Even if their sources are the same, the results show that there are variations between the tumsa and wolki variants. Thus, it appears from this study's results that the wolki variety was smaller than the tumsa. This observation is consistent with (Mitiku and Wolde, 2015) analysis, which lists the shortest and

longest heights for various fababean crop varieties. According to Talal and Munqez (2013), faba bean accessions had a substantial impact on plant height. (Della, Gangodawila and Pigou, 1988) discovered that in rain-fed conditions, genotypes of faba beans varied greatly in plant height. In other way, seed collected from HARC and ESE of wolki variety including, tumsa from OSE had the broad leaf whereas tumsa from HARC was the narrow leafed from other treatment combinations. One variation from the two seed sources scored the highest for the leaf width attribute, which may indicate that its genetic makeup caused it to have more leaves overall than the left variety. Authors (Daur, Sepetoğlu and Sindel, 2011) found that slow leaf senescence caused an increase in plant surface expansion from planting to flowering, which was subsequently followed by a decrease until seeds were ready. These findings may lend support to this theory. While there were statistically significant changes between each treatment, the number of flowers, color of flowers, and length of leaflets did not exhibit many numerical differences. Number of flower, flower color and leaflet length was doesn't showed much differences numerically however, statistically there is some significance differences among each treatments. Generally all growth traits showed up and down conditional for seed collected from different sources and have different seed classes. This could be due to management techniques used during production as well as the genetic influences of each type.

Table-2: Growth traits of different class of fababean varieties collected from different sources

Growth traits								
TRTS	Days to emergency	Plant height	Leaf width	Number of flower	Flower lenght	Leaf let lenght	Num. of leaflet/leaf	Num. of node/plant
T1	9.67a	127.47a	2.96ab	4.12b	3.62ab	T1a	T1a	26.95a
T2	9.67a	121.13ab	2.81ab	4.43ab	3.74ab	T2ab	T2a	26.93a
T3	8.67ab	119.67ab	2.69b	4.53ab	3.82ab	T3ab	T3a	26.03a
T4	9.33a	129.4a	3.06a	4.63a	3.77ab	T4ab	T4a	26.83a
T5	8.33ab	118ab	3ab	4.63a	4.52a	T5b	T5a	25.87a
T6	7.67b	118.73ab	3.1a	4.63a	3.66ab	T6ab	T6a	27.73a
T7	8.67ab	112.43b	3.11a	4.63a	3.46b	T7ab	T7a	25.57a
T8	9.33a	114.37b	2.95ab	4.37ab	3.54b	T8ab	T8a	26.1a
Mean	8.92	120.15	2.96	4.50	3.77	10.29	5.60	26.50
LSD	1.35	12.01	0.32	0.43	0.95	1.08	0.30	2.96
CV	12.87	8.47	9.06	8.04	21.35	8.91	4.50	9.48

5.2. Yield traits

The results of the ANOVA showed that practically all of the fababean's yield-related features were significantly impacted by the treatment and the combination of the factors, but that there was no significant variation in grain yield (Table 3).

The number of pods per plant in seed cultivated from the Wolki variety acquired from HARC was found to be higher, whereas the number of pods per plant in seed obtained from Ethiopian seed enterprise of the Tumsa variety at the basic and prebasic class levels was found to be lower. The pre-basic seed variety from ESE called Tumsa had the maximum number of pods per node when produced, while the same variety from HARC and OSE had the lowest values for this feature. The fact that the two values were recorded from the same variety of distinct seed source suggests that there was a difference at each source's point of seed production. The quality of seed produced varies depending on where it is produced and who is producing it. This could be due to a lack of knowledge or meticulous application of recommended practices. In another scenario, when it came to pod length attributes, the Tumsa variety from OSE had the highest value (6.68 cm), while the Wolki variety from the same location had the lowest values (5.92 cm). This conclusion allows us to acquire variation in treatments gathered from different varieties at the same place; this variation may be attributed to differences in the quality levels between the varieties.

The highest values for the attribute of seed per pode were found in plants growing from wolki variety seeds gathered from Holeta and ESE, while the lowest value was found in tumsa from ESE. This suggests that variances in values could result from variations in varieties rather than variations in the quality level throughout production, even when the seed source is the same. The primary method by which we may distinguish one variety from another is by counting off types in morphological visualization; hence, the primary goal of this work is to assess genetic purity in growth out test results, which demonstrated that the number of off-types exhibited statistically significant differences.

While the other treatment did not show such a significant numerical difference, the tumsa variety seed obtained from Ethiopia Seed Enterprise reported the highest value (12). This implies a fairly constant quality level for each treatment. The variables have no effect on the grain yield of any combination of treatments. This observation is at odds with findings from (Derese, 2022) and (Gereziher, Lemma and Molla, 2018) who found considerable variations in the tested Faba bean types' seed output (kg/ha). Similar findings were made by (Kindie and Nigusie, 2019) who found that variety

Dosha had the highest mean yield (2197.9 kg/ha). Because it affects both food security and economic effectiveness, crop yield is one of the most significant agronomic features (Donckier *et al.*, 2004). Unfortunately, despite the passage of two years, the study's grain yield did not significantly alter. It is possible that this is due to the tested variety's consistent yield potential in the testing area.

Table-3: Yield and yield related traits of different class of fababean varieties

Yield traits								
TRTS	Num. of pod/plant	Num. of pod/node	Pod lenght	Num. of seed/pode	Branch number	Off type	Grain yldkg /plot	Thousand seed weight
T1	9.07c	1.58a	6.3bc	2.25b	5ab	6.17c	1.83a	668.17a
T2	9.33c	1.57ab	6.52ab	2.47ab	6a	12a	1.83a	713.73a
T3	10.93abc	1.27b	6.16bcd	2.43ab	5ab	7.5bc	1.73a	683.7a
T4	10bc	1.27b	6.68a	2.43ab	4.33ab	6.67bc	1.81a	695.97a
T5	12.47ab	1.33ab	5.92d	2.43ab	4.67ab	8.83b	1.67a	582.13b
T6	13a	1.37ab	5.89d	2.5a	3b	6c	1.65a	554.27b
T7	12.13ab	1.53ab	5.96cd	2.33ab	3.33ab	5.67c	1.66a	553.17b
T8	12.4ab	1.37ab	6.11cd	2.53a	4.67ab	6.5bc	1.72a	581.8b
Mean	11.17	1.41	6.19	2.42	4.50	7.42	1.74	629.12
LSD	2.65	0.31	0.37	0.22	2.90	2.37	0.43	70.23
CV	20.14	18.91	5.04	7.60	14.71	27.15	21.21	9.47

6. SUMMARY, CONCLUSION AND RECOMMENDATION

One option for diversity research for highly heritable traits is morphological markers, which don't require complex laboratory setups or methods for describing the germplasm. Repeated trials conducted over several years would be an excellent way to increase the repeatability of phenotypic characterization. To examine the purity level of several fababean seeds, a two-year experiment was conducted at the Holetta research experimental site. The study involved using fababean crop seed that was gathered from various seed growers across the nation. The results showed that, overall, seed collected from Ethiopian seed enterprises, led by seed grown at Holetta, registered the best result from other treatment combinations for most traits. This suggests that, from a morphological point of view, seed collected from HARC highland pulse team (breeder seed) and some classes of seed grown by ESE showed improved purity level as compared with other kinds. Better results at distinct seed classes and kinds were also shown by the level of purity for this acquired from OSE. The optimum method, according to this study, is to use finger print evaluation to determine the purity degree of seed produced from various campanies using DNA sequencing. The reason for this is the up-and-down outcomes of morphological tests on seed that was gathered at each seed level from all sources. Testing the genetic purity of faba bean variants gathered from various sources was the goal of this investigation. The findings of the analysis of variance revealed that while certain attributes showed non-significant changes, each treatment combination's growth and yield traits shown substantial differences. However, as they record related data for each attribute numerically, it is insufficient to evaluate the purity level of seed acquired from various sources. Particularly, a significant percentage of off-types did not record significantly different outcomes for every combination of treatments. This suggests that the morphological visualization of this crop alone is insufficient to distinguish between the two varieties' levels of purity. For this reason, it is advised that this procedure be done following a marker analysis of both varieties in order to confirm their genetic purity via DNA testing. Therefore, the best course of action must be to use crop gene extraction to further investigate the genetic purity of each treatment.

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