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Table of Content

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Full Length Research Paper

Analytical performances description of the immunoturbidimetric method for the determination of HbA1c using Selectra Pro M automated system at the Institut National d'Hygiène (INH) of Lomé

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HbA1c is the best indicator for monitoring glycemic control in both type 1 and type 2 diabetic patients. Its value allows us to predict the development of long-term degenerative complications of diabetes. Several techniques offered by different laboratories allow the determination of HbA1c based on different principles. The purpose of the present study was to perform the verification of the analytical performances of the Selectra Pro M automated system, using immunoturbidimetry as the HbA1c assay method. Risk assessment of the method was performed; the analytical performances of the assay process was evaluated and the immunoturbidimetric method on the Selectra Pro M was compared to the capillary method on the Capillarys 2 Flex Piercing. Reproducibility and intermediate precision were satisfactory with CV in the range of 1.78 to 1.96% for the low level of Internal Quality Control (IQC) (HbA1c = 5.4%) and 0.82 to 2.28% for the high level of IQC (HbA1c = 11.3%). The reagent was linear from 2.5 to 16% (4 to 151 mmol/mol). The accuracy was considered satisfactory. The linear regression calculation (least squares line) showed an excellent correlation (R^2= 0.96) between the two techniques with an equation of the type [Capillarys] = 1.13 [Selectra Pro M] - 0.83. All documented and tested performances correspond to the performance required for HbA1c assay at INH of Lomé.

Key words: HbA1c, diabetes, performances of method, immunoturbidimetry, INH-Togo.

INTRODUCTION

Glycated hemoglobin (HbA1c) represents 4 to 6% of total hemoglobin. It results from the condensation of a glucose molecule with the N-terminal valine group of each of the two beta chains of hemoglobin A (Schnek and Schroeder, 1961). HbA1c represents a powerful tool for monitoring long-term glycemic control (Goldstein and Little, 1997; Vassault et al., 2010). In diabetics, a high concentration of HbA1c is associated with a wide variety of complications.

Its dosage is therefore essential to evaluate the management of diabetic subjects and the dosage technique used must be high-performance. The interest generated by this determination is at the basis of the diversity of its assay techniques having different reference value. This variability between different techniques means that the performance of the technique to be used must be verified before it is adopted. The analytical performances verification of a technique consists of evaluating the performance of the analytical process, quantifying it by following a standardized operating protocol and then evaluating it against defined criteria (COFRAC, 2015). In order to meet the requirements of its customers and bring out reliable results, the Institut National d'Hygiène (INH) of Lomé started implementing in 2002 a Quality Control process with the major commitment to be part of a logic of continuous improvement of its services. Since March 2011, special focus is placed on the Medical Biology Laboratories (MBL) for the accreditation according to ISO 15189 standard. All critical equipment newly acquired at the biochemistry laboratory of the INH is submitted to an on-site verification of all parameters available according to ISO 15189. However, some parameters including HbA1c have not yet gone through this process. In order to contribute to the improvement of the analytical performances, we initiated this study with the overall objective of verifying the analytical performances of the newly installed Selectra Pro M automated system that works on the immunoturbidimetric principle to measure HbA1c. The risks involved in applying the HbA1c assay method were assessed; then, the analytical performances of the assay process was evaluated; and finally, a comparison of the immunoturbidimetric method on Selectra Pro M to the capillary method on Capillarys 2 Flex Piercing was done.

METHODOLOGY

Assay techniques

Samples

Sixty-four whole blood samples collected on EDTA tubes (5 ml) were randomly selected. Samples were from individuals having hemoglobin A on the chromatographic profile.

Principles of measurements

Immunoturbidimetry on Selectra Pro M (ELI Tech Group, Puteaux, France): On Selectra Pro M, HbA1c assay technique is based on the principle of immunoturbidimetry and the HbA1c level is calculated from a non-linear calibration curve obtained from four standards of different levels and a zero point. Two control levels, low and high (ELI Tech Control L+H), are assayed to each series.

Capillary method on Capillarys 2 Flex Piercing (SEBIA, Lisses, France): On Capillarys 2 Flex Piercing, HbA1c measurement is based on the principle of capillary electrophoresis in free solution. It allows the separation of charged molecules according to their own electrophoretic mobility in a given pH buffer, and according to the pH of the electrolyte, from a more or less important electro-osmotic flow. The separation is achieved by applying a potential difference of several thousand volts at the terminals of each capillary.

Technical steps of the verification protocol

The evaluation was performed in 3 time points:

(1) One week of familiarization and learning about the device;
(2) Two weeks during which repeatability tests were performed at 2 concentration levels (low, high); and reproducibility tests calculated from the results of samples tested in 2 different series per day for 10 days;
(3) Three weeks during which the comparison between the HbA1c values of the different machines was performed.

Risk assessment

The 5M method was used by considering all the critical points (strengths and weaknesses) concerning: (1) the premises and environmental conditions (layout, temperature); (2) reagents (preparation, batch-to-batch variations and stability); (3) equipment (compliance with supplier operating procedures and instructions, maintenance, calibration, metrological connection); (4) staff (training, evaluation of skills); (5) method (performance criteria: precision, accuracy, uncertainties, interferences), taking into account the quality criteria of the samples analyzed.

Evaluation of the performance of the assay method

The analytical evaluation protocol was inspired by COFRAC's SH-GTA 04 (4) reference protocol.

Repeatability assessment: For the repeatability assessment, 20 assays were performed in the same series, on the same day, with the same procedure, the same operator, the same batch of reagent and the same working conditions, with two levels of control. The coefficient of variation (CV) was used to evaluate the repeatability of the method expressed as a percentage. CV was calculated using this formula:

$$m = \frac{\sum x_i}{n} \qquad S = \sqrt{\frac{\sum (xi - m)^2}{n-1}} \qquad CV\ en\ \% = \frac{(S) \times 100}{m}$$

where m is the mean value of Hb1Ac measured, n is the number of

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tests, S is the standard deviation.

This calculated CV was compared to the admissible limit CV given by suppliers or learned societies such as Société Française de Biologie Clinique (SFBC).

Assessment of the intermediate precision: The intermediate precision was determined from the results obtained on control specimens at two concentration levels, assayed daily in 2 different series per day for 10 days, by varying the operating conditions (operator, calibration, batches of reagents, etc). The calculation methods were similar to those for repeatability. The CV calculated on the experimental values of each series is compared to the admissible limit CV.

Accuracy approach: The accuracy, quantified by bias, was estimated by comparing the mean (m) to the expected target value assimilated to the true value (v). The m value is obtained during the intermediate precision study (intra-laboratory reproducibility) and established with samples of internal quality control. The bias is expressed as a percentage of the target value.

$$\text{Bias (\%)} = 100\ x\ \frac{(m-v)}{v}$$

Comparison of methods: The accuracy of the immunoturbidimetric technique was assessed against the Capillary technique. To do this, 64 individuals' whole samples were analyzed on the two devices in a short time. The comparison between the two methods was made using 4 tools:

(1) The graph of ratios of the values from Selectra to those from Capillarys;
(2) The equation of the regression line according to the method of least squares and the determination of the regression coefficient;
(3) The analysis of the diagram of the differences between the 2 techniques according to the instructions of Bland and Altman.
(4) The t test of the differences to see if the differences observed between the results are statistically significant. The calculated t was compared to the theoretical t with (n-1) DOF (n is the number of tests and DOF the degree of freedom).

Statistical analysis

The repeatability, reproducibility and bias data were entered and analyzed in Microsoft® Excel 2010 spreadsheet. The comparison of the methods was made with the R 3.3.1 software. The differences were considered significant at a value of P less than 0.05 (P<0.05).

RESULTS AND DISCUSSION

Risk assessment

The results of the risk assessment using the 5M method showed ten strong points and seven weak points which are presented in Table 1.

Performance evaluation

Repeatability of Selectra Pro M

The CV from the repeatability calculation for low and high IQC was 1.78 and 0.82, respectively (Table 2). These obtained CV are lower than the supplier and SFBC CV. We then deduced that our repeatability data are consistent.

Intermediate precision

The low and high IQC CV are 1.96 and 2.28, respectively. These CV are lower than the supplier's and SFBC's CV. It can then be inferred that the intermediate precision data on the Selectra Pro M are compliant (Table 3).

Approach to accuracy

The biases of the low and high IQC are 0.92 and 0.044, respectively, and are lower than the SFBC accuracy values (Table 4).

Comparison of Selectra Pro M and Capillarys methods

The reports graph: The graphical representation of the Selectra/Capillarys ratios (Figure 1) shows a homogeneous distribution of results over all the HbA1c values studied and are in the range [0.8; 1.2], therefore close to 1.

Regression line or Passing-Bablock for Selectra Pro M and Capillarys methods: The regression line shows a good correlation between the two methods (Figure 2).

Bland-Altman graph: Good agreement was obtained for HbA1C values below 16% (Figure 3). The mean difference md= -0.19%. Most of the points are in the range [-1.41; 1.02], the range of agreement limits (md ± 2sdd). Thus, of the 64 values compared, only 3 are outside the approval limits.

The difference t-test

The t-test of differences was performed on the two sets of results given by the two devices. The mean of the differences (md) was calculated. The calculated t was compared to the theoretical t with (n-1) DOF. Table 5 shows the data of the t-test of differences. The results of the two analytical systems therefore showed statistically significant differences at the 5% risk threshold (Table 5).

DISCUSSION

This study was initiated in order to verify the analytical performances of a newly installed Selectra Pro M automated system that uses the immunoturbidimetric

Table 1. Risk assessment with the 5M method.

5M	Critical points	Strong points	Weak points
Matter (reagents)	Storage and conditions of use Reagents and standards reconstitution	Good reagents storage measures Compliance with the operating procedure and reagent supplier manual	Failure to calibrate the micropipettes periodically Lack of metrological traceability
Medium	Local Environmental conditions	Good metrology and monitoring of enclosures Static environmental conditions over time	Recording of environmental conditions not updated
Material	Drift monitoring Contamination	Compliance with supplier's operating procedures and instructions. Periodic maintenance, calibration	Operating procedure not written for HbA1c
Method	Performance criteria Cause of measurement uncertainty	On-site verification for some parameters ; Compliance with non conformity management procedures	No on-site verification for HbA_{1c} Lack of calculation of measurement uncertainties of quantitative parameters
Manpower (Staff)	Skills and skill maintanance	Training planning and staff assessment Authorized personnel for HbA1c dosing.	Lack of a personnel evaluation grid for HbA1c testing

Table 2. Repeatability study of Selectra Pro M.

Sample (IQC)	Number of values (n)	Mean (m)	Standard deviation	CV (%)	CV (%) supplier	CV (%) (SFBC)	Conclusion
IQC Low (5.4)	20	5.235	0.093	1.78	2	3.8	Compliant
IQC High (11.3)	20	11.335	0.093	0.82	3	3.8	Compliant

*Société Française de Biologie Clinique (SFBC).

Table 3. Intermediate Reliability Study of Selectra Pro M.

Sample	Number of values (n)	Mean (m)	Standard deviation	CV (%)	CV (%) supplier	CV (%) (SFBC)*	Conclusion
IQC Low (5.4)	20	5.35	0.105	1.96	2	5	Compliant
IQC High (11.3)	20	11.31	0.258	2.28	3	5	Compliant

*Société Française de Biologie Clinique (SFBC).

method to measure HbA1c. Indeed, a "scope A verification" was already carried out where the recognized methods (CEmarked IVDDs or "supplier" methods) are validated in their field of application. Our analytical performances verification of the HbA1c assay method compared

Table 4. Accuracy approach of Selectra pro M.

Sample	Results (%)	Ref Val. v (%)	Difference (%)	Bias (%)	Bias (SFBC) (%)	Conclusion
IQC Low	5.35	5.4	0.05	0.92	6.2	Compliant
IQC High	11.305	11.3	0.005	0.044	6.2	Compliant

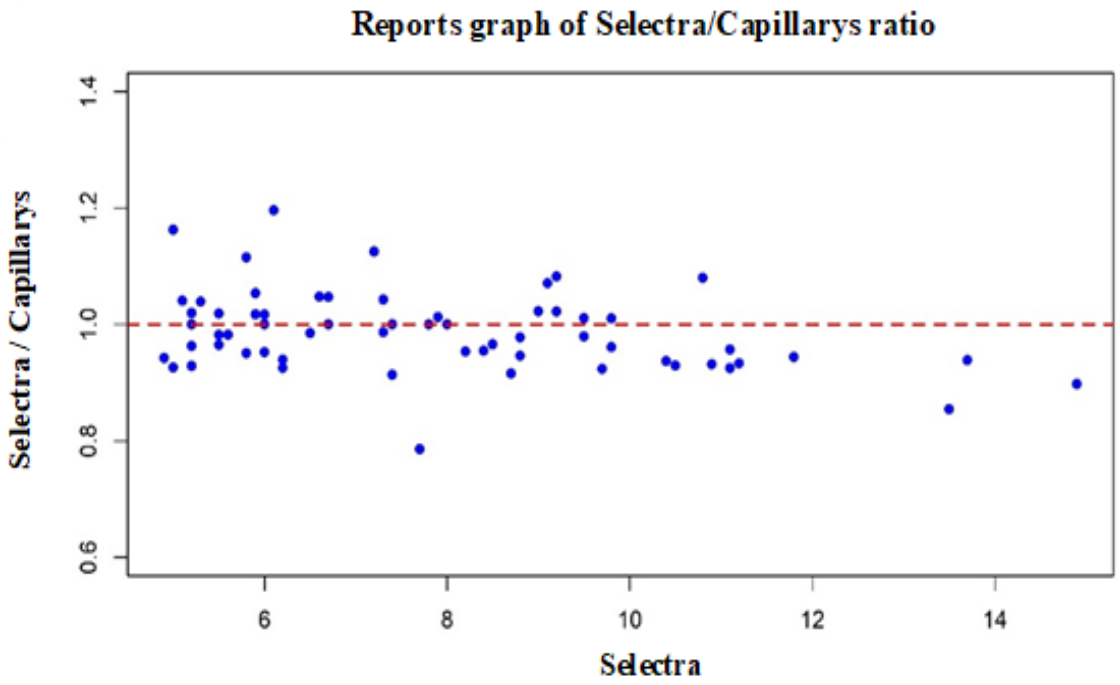

Figure 1. Representation of the Selectra Pro M/Capillarys ratio graph. The points on the graph represent the ratio values between Selectra Pro M and Capillarys. All values are between 0.8 and 1.2.

the results from Selectra Pro M to those of the mirrored Capillarys 2 Flex Piercing.

The evaluation of the risks according to the different critical points, in spite of the different strong points, still showed weak points to be improved. In particular, calibration of micropipettes and metrological traceability to ensure proper packaging and reconstitution of reagents; recording and updating environmental conditions to avoid their influence on the technique; writing the handling procedure to ensure the suitability of the material to be used; on-site verification and calculation of uncertainties to evaluate the performance of the method and having a personnel evaluation grid to ensure the qualification of the personnel.

After risks assessment, we evaluated the performance of this method by evaluating the repeatability, the intermediate precision, and accuracy assays.

The CVs for the repeatability study complied and generally met the requirements issued by the supplier and also the criteria of the VALTEC protocol (SFBC). Similarly, the CVs achieved were similar to those of Beaune et al. (2009) with CV ranging from 1.18 to 1.91% in the evaluation of a technique for the determination of HbA1c on Architect CI8200 using an immunoturbidimetric technique. However, Samaan et al. (2007) and Urrechaga, (2018) found CVs of less than 1% whatever the level measured and whatever the material used (fresh blood or lyophilized control), CVs of 0.42 to 0.30% and 0.71 to 0.43%, respectively. This difference can be explained by the technique used (HPLC), which is a much more precise technique.

The CVs for the intermediate precision study showed reproducibility in accordance with the supplier's requirements. These CVs are consistent with those of Beaune et al. (2009) and El Arabi et al. (2013) in the evaluation of DCA Vantage which used an immunological agglutination technique for the determination of HbA1c and found CVs ranging from 2.09 to 2.64% and 0.9%, respectively, while Urrechaga, (2018) found more satisfactory CVs of 0 to 0.36%.

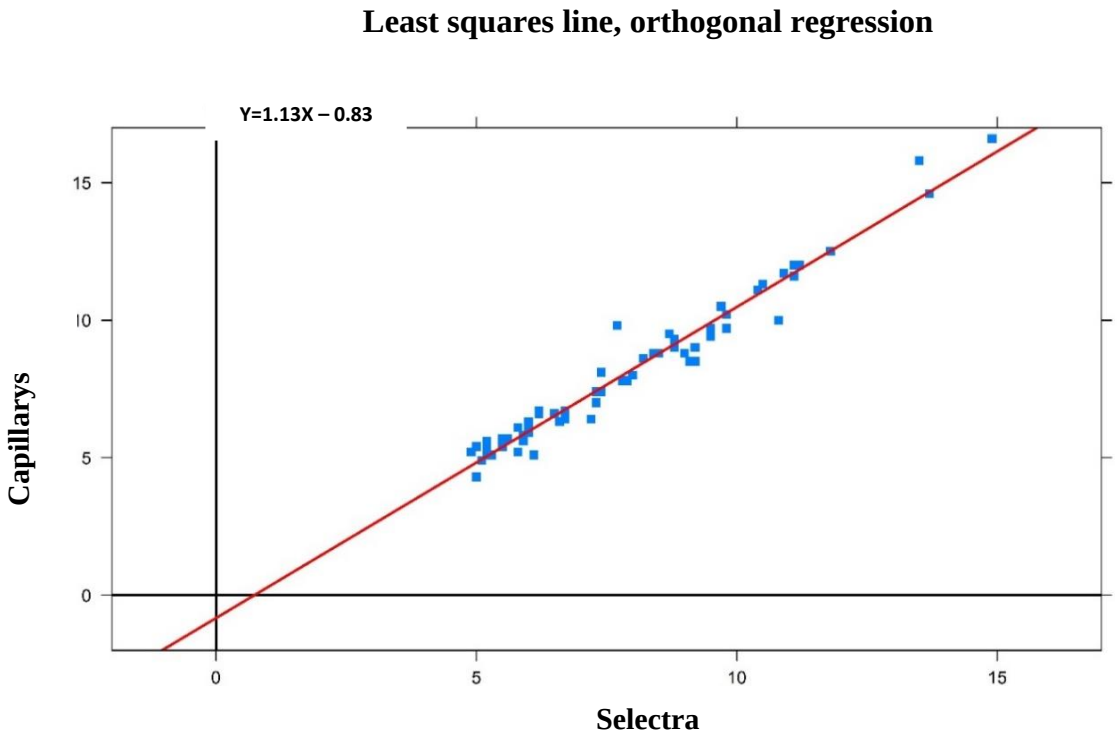

Figure 2. Regression between Selectra and Capillarys values. The right side of the graph represents the correlation of the intercept capillary technique on the Capillarys versus the immunoturbidimetric technique on Selectra Pro M. Intercept at origin (Intercept) = -0.83, Regression coefficient (R^2) = 0.96.

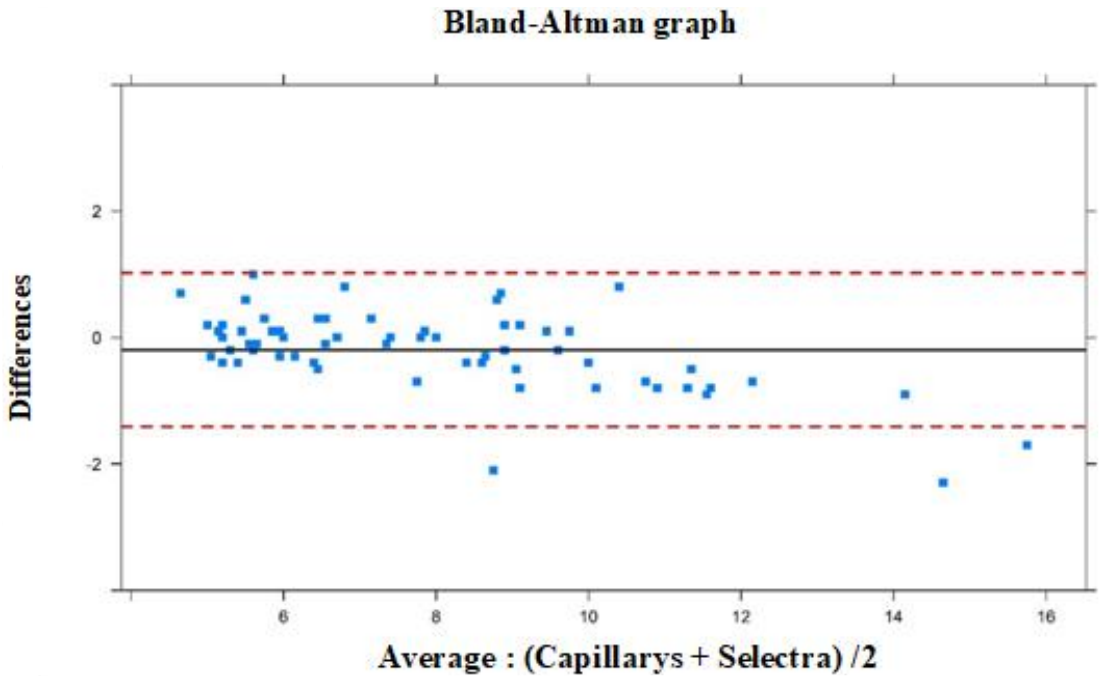

Figure 3. Representation of the Bland-Altman graph of Selectra Pro M and Capillarys methods. The points on the graph represent the average of the differences in the values of the two methods. Only 3 points are outside the approval limits.

Table 5. Data from the t-test of differences between Selectra Pro M and Capillarys.

Variable	Analytical system		Difference
	Selectra Pro M	Capillarys	Selectra-Capillarys
Average (m_d)	7.82	8.00	-0.19
t calculated	2.55		
DOF	63		
p (value)	0.01		

During a method evaluation, the measurements may be close to each other (good precision) but outside the probability range of the true value, they are therefore biased (bad precision) or vice versa. However, a method is said to be accurate if it is both faithful and fair. In view of the level of biases obtained in this study and thus compared with the SFBC specifications, the immunoturbidimetric method is considered accurate.

The relevant data brought by the equation of the regression line whose slope (1.13) and intercept at the origin (-0.83) express the similarity of the methods compared. Beaune et al. (2009) correlated Architect Abbott (Immunoturbidimetry) with HPLC D-10 Bio-Rad and found comparable results (R^2 = 0.98 for 161 samples tested) with a regression line of equation [Abbott] = 1.02 [Bio-Rad] -0.636. Grant et al. (2017) and Berlanda et al. (2020) found also the same R^2=0.96 in respectively making comparison between D10 and Quo-Test with an equation of the type: Quo Test = 0.94 [D10] +4.93 and the evaluation of an automated immunoturbidimetric assay for detecting canine C-reactive protein.

The mean difference (md= -0.19%) indicates that the results obtained with the Selectra Pro M are slightly lower. Several studies have reported interferences between the other variants of hemoglobin when measuring the HbA1c (Little et al., 2008; Little and Roberts, 2009; Lee et al., 2011). This decrease of up to 1.41% (-2sd) in the results is explained by the fact that an underestimation of HbA1c was reported with immunological tests in the presence of high fetal hemoglobin concentrations as stated (Adekanmbi et al., 2016). The t-test of difference questions the transferability between the two (2) methods. It is commonly accepted in the diabetes community that a 0.5% difference in HbA1c empirically reflects a 1 mmol/L (approximately 0.25 g/L) change in mean blood glucose levels over the last 120 days prior to sampling (Simmons and Hlaing, 2014). Such variations for HbA1c values of less than 8% may lead to erroneous changes in the treatment of the diabetic (1). Intensification of therapy may be associated with side effects such as hypoglycemia with all its consequences (Seaquist et al., 2013) and a falsely assumed positive trend in HbA1c could be detrimental to the patient through continued poor metabolic control (Roth et al., 2018).

Conclusion

The assessment and control of risks in the context of this on-site verification enabled the implementation of the necessary actions to reduce and/or eliminate the potential risks identified. The performance criteria evaluated (repeatability, reproducibility and the approach to accuracy) are in accordance with the requirements of the supplier and the SFBC learned society and therefore reliable and fair. When comparing the two methods, despite the good correlation between them, there are still statistically significant differences at the 5% risk level. Therefore, even though both techniques have good accuracy, it is important to always follow the patients with the same technique or to be aware of the differences. All performances documented and tested meet the performance requirement of HbA1c assay. The immunoturbidimetric method is therefore declared suitable for the HbA1c assay.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Typing of enterovirus identified from Moroccan mussels (*Mytilus galloprovincialis* Lamarck, 1819) by seroneutralization

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Non-poliovirus enteroviruses (NPEV) are infectious agents which can determine various illness in human such as hand-foot-mouth syndrome, angina, respiratory diseases, acute or chronic heart disease, diarrhea, pancreatitis, acute hemorrhagic and conjunctivitis. These viruses are eliminated in the stool and thus contaminate the marine environment and shellfish. In Morocco, shellfish sanitary quality analysis does not include enteroviruses detection. Therefore, the objective of this study was to detect and to type enteroviruses in 288 mussel samples. These samples (*Mytilus galloprovincialis*) were collected between February 2014 and February 2015 from three wild populations (Bouregreg estuary, Yacoub Al Mansour, and Harhoura coast). 216 of 288 samples (75%) were revealed positive by the cell culture method, with 204 strains of NPEV (70.8%) and 12 strains of *Poliovirus* Type 1 (4.2%). The serotype of 204 NPEV strains has been determined a typable strains (64.7%) and non-typable strains (35.3%) in the marine environment. However, the proportion of untypable strains confirms the presence of new serotypes. The diseases caused by NPEV constitute an important public health problem. To fight against this human risk related to viral contamination, it is necessary to have a methodology for the control and virological monitoring of the marine ecosystem.

Key words: Non-poliovirus enteroviruses, *Mytilus galloprovincialis*, marine environment, shellfish, seroneutralization.

INTRODUCTION

The microbial pollution in the marine environments is a key determinant for the evaluation of the level of viral contamination, with major impacts on the control of the faecal risk for human health. For evaluating the latter risk, different markers have been proposed, including enteroviruses and adenoviruses (Hot et al., 2003; Rajtar et al., 2008; Fong and Lipp, 2005; Jung et al., 2014; Warish et al., 2018).

The advantage of enteroviruses as a marker of viral contamination is that certain genotypes are relatively easy to cultivate in cell culture, which is still the reference method for environmental monitoring (Ehlers et al., 2005; Hematian et al., 2016; Itani et al., 2023). Indeed, typing enteroviruses strains existent in the marine environment may be an important objective, especially to detect the presence of non-poliovirus enteroviruses strains and

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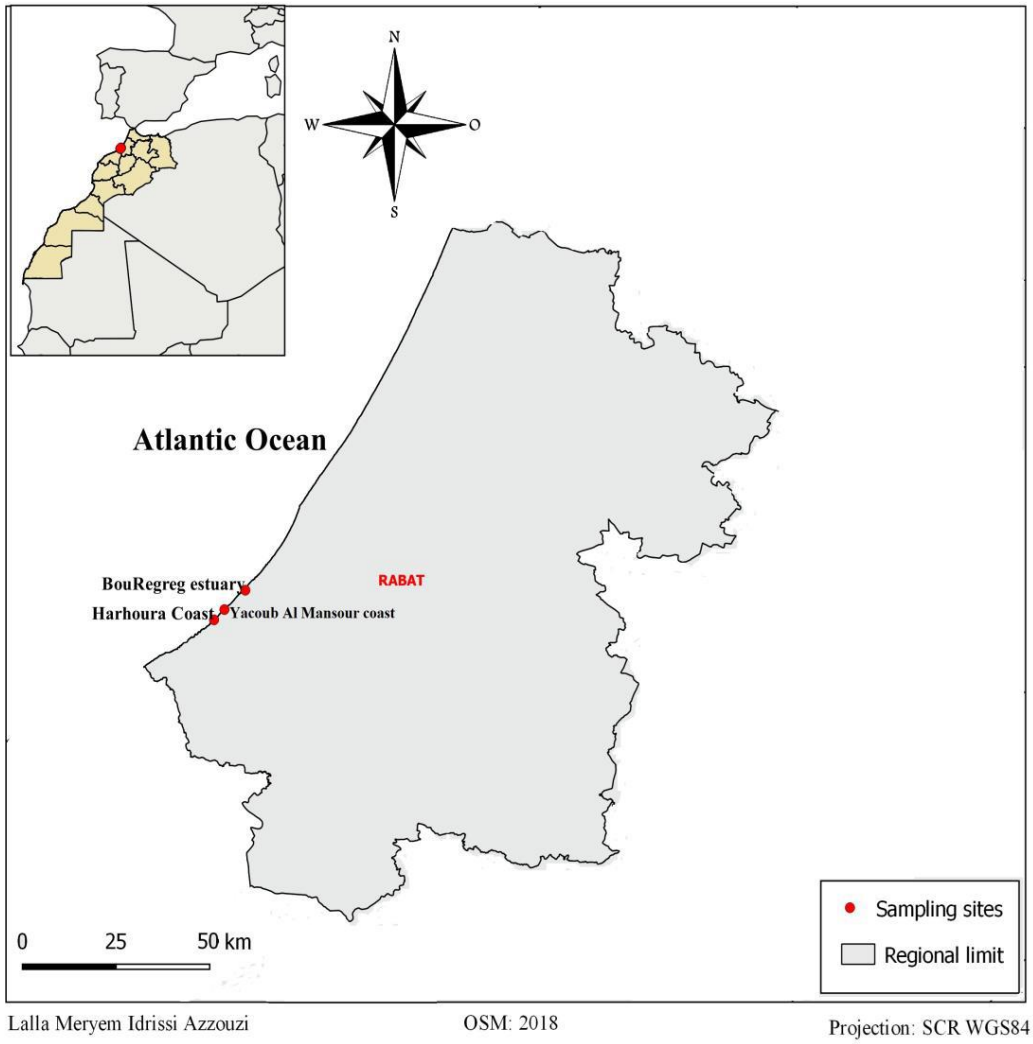

Figure 1. Map of Morocco coast showing the sampling sites.

Poliovirus strains in areas where these agents are still circulating (Hovi et al., 2005; Klapsa et al., 2022).

Non-poliovirus enteroviruses circulate in all populations and infection can be associated with a vast range of presentations. In this study, the serotype identification of non-poliovirus enteroviruses was done according to the procedures recommended by the World Health Organisation (WHO, 2004).

The identification of newly isolated strains by specific neutralization becomes increasingly difficult, as many types of enteroviruses exist in the environment. Seroneutralization tests with composite antiserum pools are very economical in tissue culture and time that the use of pooled antiserum initially is advantageous. The reference method for the laboratory diagnosis of enteroviruses is isolation of the cell culture, followed by serotype identification (Oberste and Pallansch, 2005; Hambling et al., 2009).

The lack of a national monitoring program of Enteroviruses was one of the reasons for this study in order to evaluate the viral contamination in mussels collected from potentially polluted areas. The target of this research was to study circulating strains of enteroviruses in the marine environment, in order to supplement the Moroccan databases available on environmental contamination by enteroviruses and to illustrate the importance of including routine virological analysis of shellfish in Morocco.

MATERIALS AND METHODS

Study area

Between February 2014 and February 2015, three sampling sites (Bouregreg estuary, Yacoub Al Mansour and Harhoura coast) located in the Rabat Region of Morocco (Figure 1) were chosen for the collection of 288 mussel samples (*Mytilus galloprovincialis*) from wild population sites that receive domestic waste without

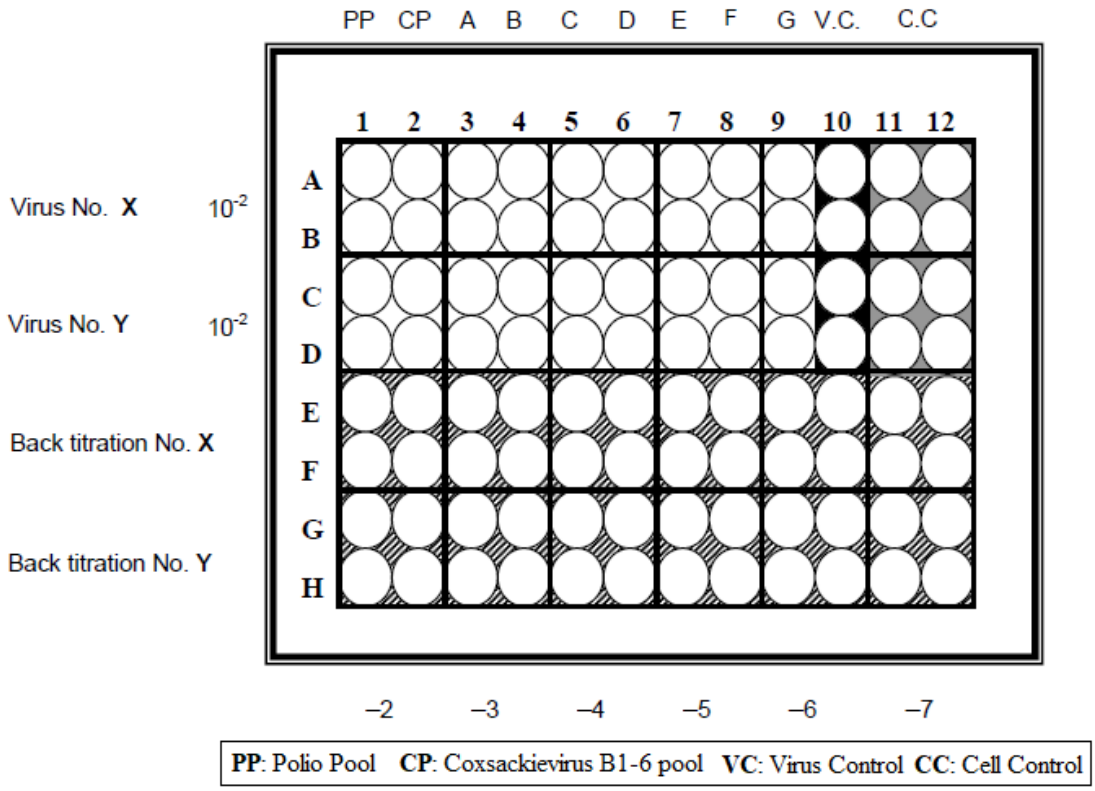

Figure 2. Plate set-up for identification of Enterovirus isolates using seroneutralization.

previous treatment. This region covers an area of 18.194 km^2, with a population of about 4.581.000. This area belongs to the Mediterranean climate characterized by two main seasons softened by oceanic influences. The average temperatures are approximately 22°C for the warmer months (July to September) and 12°C for the colder months (December and January). Relating to the annual rainfall is in average more than 550 mm/year (Idrissi Azzouzi et al., 2017a, b).

Samples preparation

The shells were opened aseptically; the digestive system was dissected with a sterile knife allowing the elimination of inhibitors tissues (polysaccharides, sexual gonads). To analyse a larger number of individuals 1.5 g of hepatopancreas were used with a weight corresponding to an analysis, representing on average 12 mussels.

Virus recovery from mussels' samples

Two hundred and eighty-eight samples of mussels were collected from three stations of Rabat Region in Morocco. Mussels samples (1.5 g of hepatopancreas) were added to 10 mL of buffer (0.1 M glycine: 0.3 M NaCL) at a pH = 9. The mixture was homogenized for 15 min then centrifuged at 10 000 g for 10 min at 4°C. The pellet was resuspended in 5 mL of phosphate buffered saline (PBS) and adjusted to pH = 7.

The mixture was homogenized again and centrifuged at 10 000 g for 30 min at 4°C. The supernatant was used for virus detection.

Concentration of virus suspensions

Virus particles recovered from mussel samples by precipitation with polyethylene glycol (PEG) 6000 at 50% as previously described (El-Senousy et al., 2013; Idrissi Azzouzi et al., 2017a). In brief, suspensions were mixed with 25% (*V/V*) PEG 6000 and incubated at 4°C overnight. The mixtures were then centrifuged at 10 000 g for 30 min. The final pellet was resuspended in 5 ml of 0.1 M phosphate buffer pH 7.2 and then filtered through a 0.22 µm Millex-GS membrane. To prevent the contamination of the concentrate, it was necessary to add to the mixture 30 µL of antibiotics (Penicillin 10 000 U/mL and Streptomycin 10 000 µg/mL) and 20 µL of Fungizone (250 µg/mL). The suspension was either treated immediately or stored at -20°C until use.

Typing of non-poliovirus enteroviruses with antiserum pools

Poliovirus strains have been identified by molecular method (real-time PCR) according to the procedures recommended by WHO (Idrissi Azzouzi et al., 2017a).

The identification of non-poliovirus enteroviruses serotypes (serotyping of NPEV) by the seroneutralization test was done using pools of antiserum prepared and provided by the National Institute of Public Health and the Environment (RIVM) (WHO, 2011).

Each box of RIVM enteroviruses typing antiserum contains anti-enterovirus pools A, B, C, D, E, F and G, anti-Coxsackievirus B pool and a trivalent anti-Poliovirus pool (Figure 2). These pools must be diluted before use. The recommended dilution for all pools is 0.5 mL, of which each pool is added to 9.5 mL of maintenance medium (minimum essential medium MEM with HEPES and 2% FBS).

Table 1. Association of Antiserum pools (A-G) for non-poliovirus enteroviruses typing by seroneutralization.

Antiserum pool	A	B	C	D	E	F
A		*Echovirus 4*	*Echovirus 7*	*Echovirus 11*	*Echovirus 14*	*Echovirus 9*
B	*Echovirus 4*		*Coxsackievirus A9*	*Echovirus 1*	*Echovirus 27*	*Echovirus 3*
C	*Echovirus 7*	*Coxsackievirus A9*		*Echovirus 21*	*Echovirus 22*	*Echovirus 2*
D	*Echovirus 11*	*Echovirus 1*	*Echovirus 21*		*Echovirus 20*	*Echovirus 12*
E	*Echovirus 14*	*Echovirus 27*	*Echovirus 22*	*Echovirus 20*		*Echovirus 33*
F	*Echovirus 9*	*Echovirus 3*	*Echovirus 2*	*Echovirus 12*	*Echovirus 33*	
G	*Echovirus 6*	*Echovirus 25*	*Echovirus 5*	*Echovirus 30*	*Echovirus 29*	*Echovirus 13*

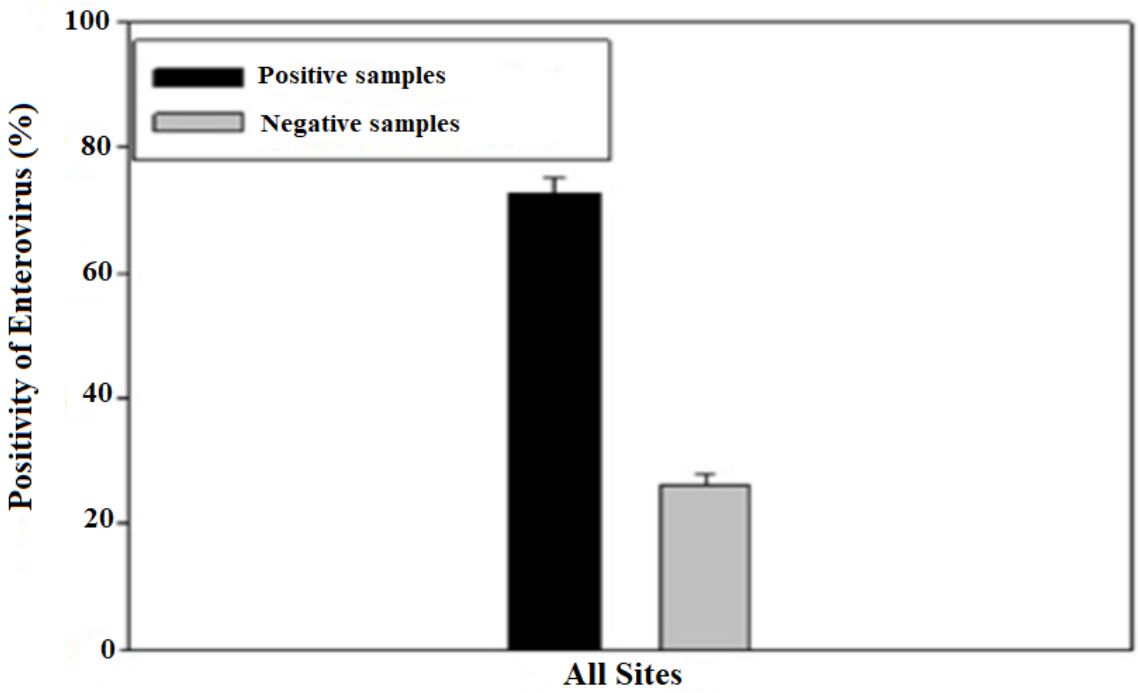

Figure 3. Percentage of positivity for enteroviruses in mussels.

Aliquot pools into clearly labelled cryovials in 1 mL volumes and store at -20°C. Each unknown virus was tested in duplicate against a trivalent-pooled poliovirus antiserum (PP), a Coxsackievirus B1 to B6 pool (CP), and seven pools against Coxsackievirus A9 and 20 echoviruses (A-G) (Figure 2 and Table 1). Non-poliovirus enteroviruses that fail to be identified using this antiserum may be in an aggregated form that interferes with the complete neutralization by specific antiserum. Isolates can be retested after emulsification with chloroform (approximately 10% by volume) and separation of the supernatant.

The virus suspension to be used in the seroneutralization tests was prepared by the inoculation of cultures of the specified cells. After inoculation, the cultures were examined daily for cytopathic effect (CPE). Complete destruction of the cells within 3 days is preferable, and if this was not obtained initially, a further passage should be made. When destruction is complete, the cultures are frozen, then rapidly thawed, and harvested. This harvest forms the stock virus suspension for all the seroneutralization tests and is stored at -20°C until required.

Because a large number of viruses makes it impractical to perform individual neutralization tests, these have been pooled in an overlapping scheme that allows many viruses to be identified using as few as nine tests. Interpretation of the results was done with the assistance of a list of the neutralization patterns of individual viruses (Table 1).

RESULTS AND DISCUSSION

The virological analysis (cell culture) of 288 samples collected from Bouregreg estuary, Yacoub Al Mansour and Harhoura coast, showed that 75% of mussels (*Mytilus galloprovincialis*) were contaminated by enteroviruses (Figure 3), with 204 strains (70.8%) of non-poliovirus enteroviruses (NPEV) and 12 strains (4.2%) of *Poliovirus* Type 1 (SL1) which was confirmed by real-time PCR using intratypic differentiation (ITD) method (Figure 4).

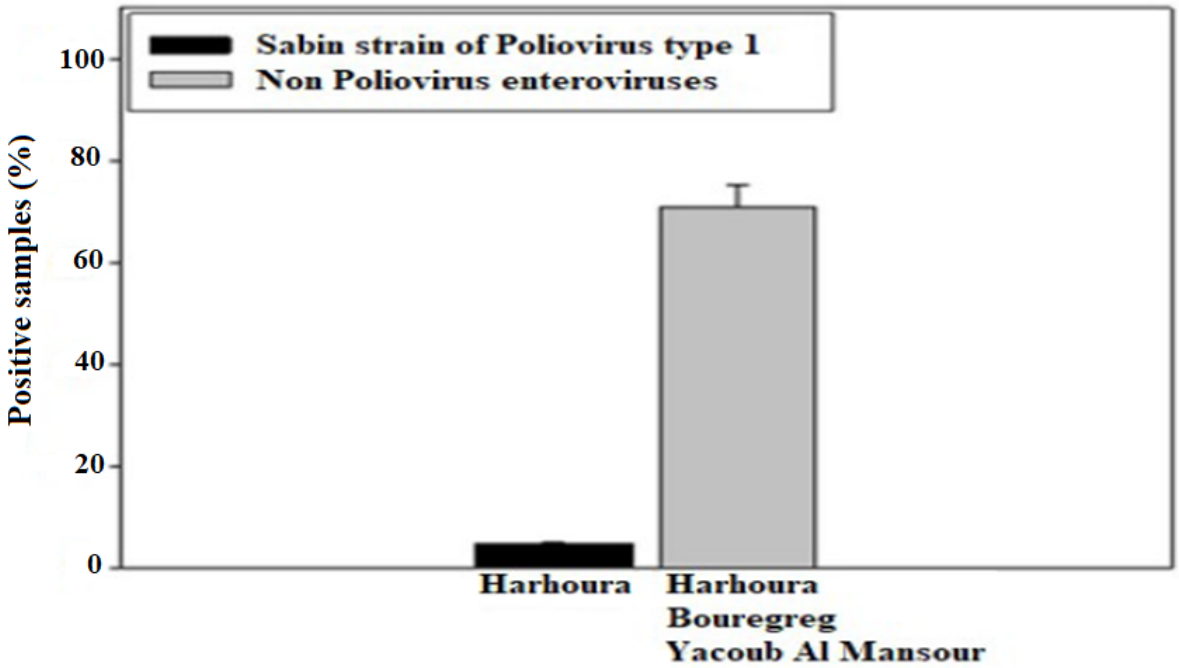

Figure 4. Percentage of positivity for Sabin strain of poliovirus type 1 and non-poliovirus enteroviruses in mussels.

From the isolates of NPEV were obtained RD cell lines, 204 of these strains were serotyped by seroneutralization using pools of antiserum, however only 132 strains (64.7%) could be identified, against 72 non-typable strains (35.3%) (Figure 5). Among these typable strains, 72.7% (96/132) have been determined as *Coxsackievirus* B and 27.3% (36/132) as *Echovirus*) (Figure 6). The different serotypes of C*oxsackievirus* B and *Echovirus* could be identified as 62.5% of *Coxsackievirus* B5 (60 strains), 37.5% of *Coxsackievirus* B3 (36 strains), and 100% of *Echovirus* 6 (36 strains) (Figure 7).

This study revealed the circulation of an important number of typable strains (64.7%) and non-typable strains (35.3%) in the marine environment. However, non-typable strains confirm the presence of new serotypes. A seroneutralization test was used for the identification of enteroviruses in tissue culture with composite antiserum pools. This antiserum with twenty-seven enteroviruses was included in the pools that were used to examine 204 of non-poliovirus enteroviruses that consist of typed and untyped strains. The results indicate that this method provides a useful screening method for identifying enteroviruses. It has proved to be practicable, time-saving, and very economical in tissue culture.

The analysis of viruses in environmental samples is complex. There are a number of issues to consider. Primary isolates of many viruses of interest grow poorly, if at all, in cell culture systems (Duizer et al., 2004; Straub et al., 2007; Tanaka et al., 2007; Cromeans et al., 2008; Gerba and Betancourt, 2019). This is further compounded by the fact that major enteric viruses are present in low numbers in the environment, and have been shown to have an infectious dose ranging from 1 to 100 particles. Therefore, a method must concentrate low levels of viral particles and eliminate any inhibitory substances that could interfere with the analytical process (Brundage and Fitzpatrick, 2006; Teunis et al., 2008). For this reason, it is important to develop methods sensitive enough to detect a single viral particle per sample. In addition, some of the important enteroviruses have a high degree of genetic and antigenic variability (Kageyama et al., 2004; Matthijnssens et al., 2008; Zheng et al., 2006). Therefore, to monitor viral contamination in marine environments, the use of molecular techniques targeting certain regions of the genome and the phylogenetic analysis of nucleotide sequences are recommended. These techniques will make it possible to identify new serotypes while ensuring the characterization of non-typable strains. Molecular tests will also, by the determination of recombinant strains explain the genetic evolution of NPEV strains.

The prevention of diseases caused by non-poliovirus enteroviruses such as Coxsackievirus B and Echovirus requires the identification of viral contamination sources and the development of effective intervention strategies and decontamination procedures for shellfish and aquatic ecosystem (Morley, 2010; Esposito et al., 2022).

Comparing the occurrence of viral pathogens in shellfish is difficult since few data are available in the literature and conditions are always different including site conditions, sampling, and detection methods. However, this study can be compared with a previous study conducted to analyse viral contamination in mussel

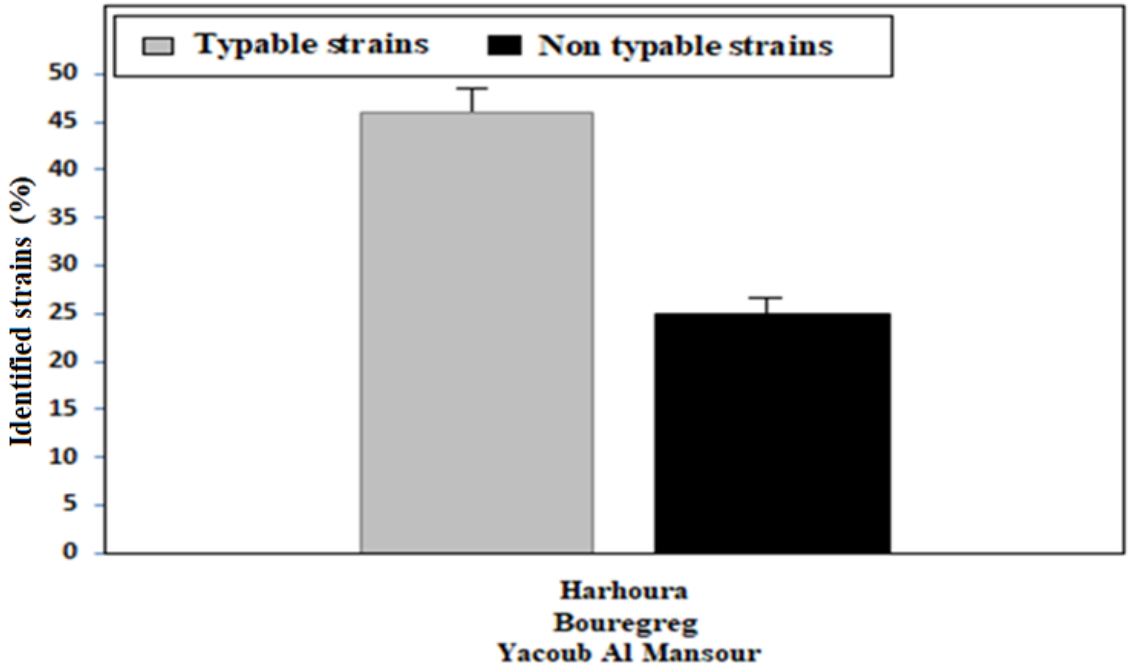

Figure 5. Percentage of typable and non typable strains of non-poliovirus enteroviruses using seroneutralization.

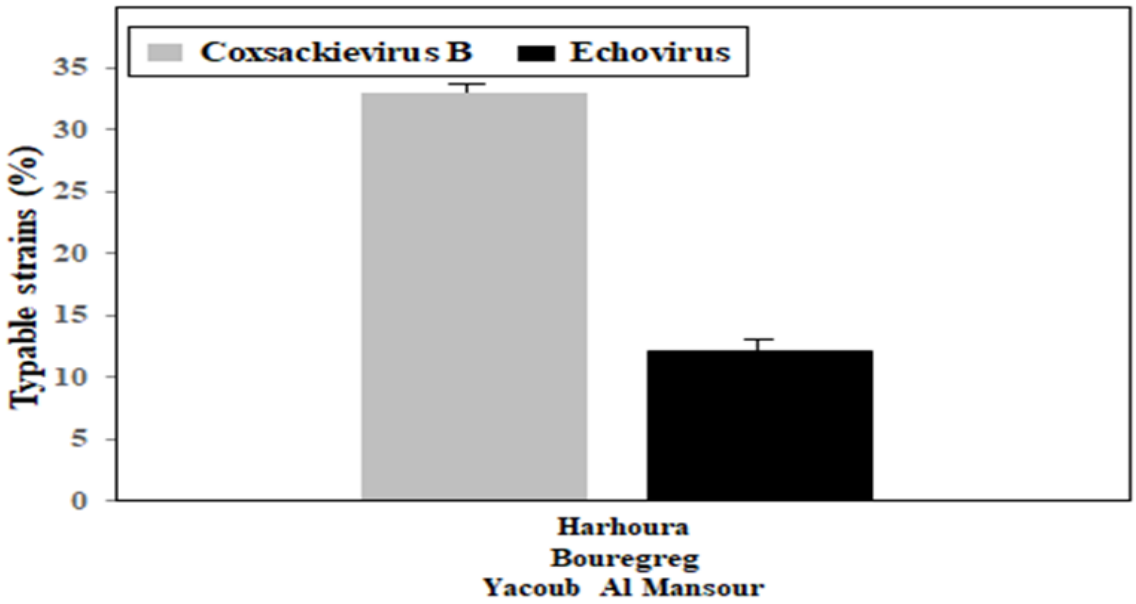

Figure 6. Typing of non-poliovirus enteroviruses strains identified from mussels.

samples collected from sites occasionally impacted with sewage (Sdiri et al., 2004, 2006; Elamri and Aouni, 2005; Elamri et al., 2006; Karamoko et al., 2005, 2006a, b; Gharbi-Khelif et al., 2007; Bosch et al., 2008; Benabbes et al., 2013a, b; Bou M'handi and Laachir, 2015; Idrissi Azzouzi et al., 2017a). These results allow some conclusions and comments, as many of the collected samples have been found contaminated with human enteric virus particles.

Conclusions

The identification of isolated non-poliovirus enteroviruses

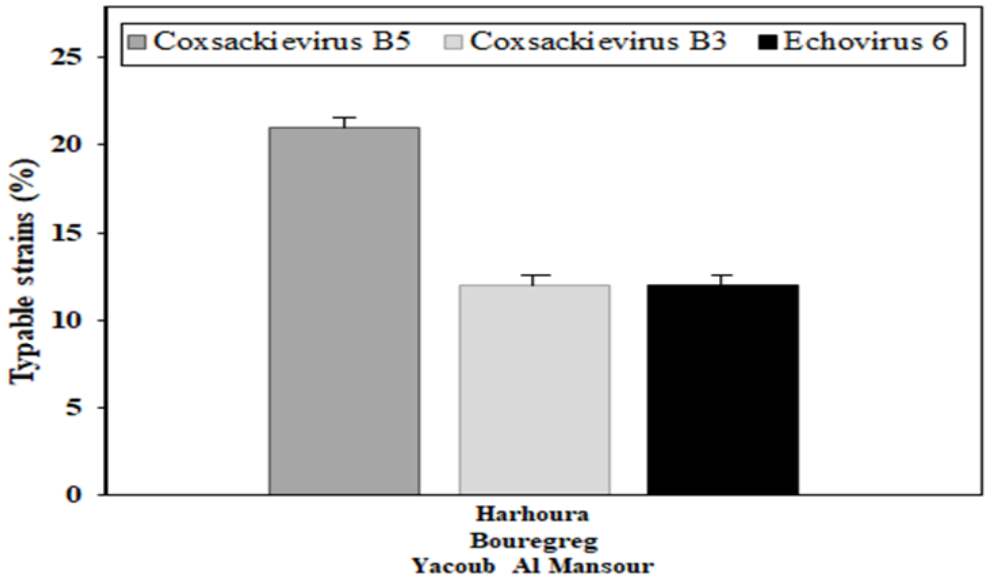

Figure 7. Percentage of different serotypes of C*oxsackievirus* B and *Echovirus* typing by seroneutralization.

becomes indispensable, as it is important to know the new serotypes associated with diseases. Thus, the surveillance of enteroviruses circulation cannot be limited to the only surveillance of interhuman circulation and should include monitoring of enteroviruses in the marine environment.

The mean number of positive samples in this study is in accordance with data found in the literature, indicating that viral contamination of molluscs is similar among countries investigated which reflects the epidemiological status of the population. The results confirm that mussels in Morocco were contaminated with several enteroviruses. Therefore, to protect human health worldwide, research should be dedicated to better understand virus circulation and to develop appropriate monitoring in all shellfish producing countries. This will be helpful to understand virus circulation and to improve seafood safety

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Morphological traits variation of cowpea (*Vigna unguiculata* L. Walp) grown in Côte d'Ivoire

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The genetic diversity of a genetic resource is an indicator of its richness and offers many possibilities for improvement. Cowpea is an important source of nutrients for humans and animals. This study aimed at assessing genetic diversity and relationships present among 135 accessions in the Centre National de Recherches Agronomiques (CNRA) collection, using qualitative traits. Traits analysis revealed significant genetic variation between the studied accessions through high Shannon diversity index. Estimated for 15 morphological characters with 0.80 mean value, H' ranged from 0.55 to 1. Seed traits generally expressed mean high diversity index (0.70-0.89). Between 15 parameters assessed, 12 exhibited the most discriminating variability of collection. The three MCA factorial axes explained 19.085% of phenotypic variability. The trait seed colour being correlated to these axes could be used as a selection criterion for local cowpeas improvement. Based on MCA and HAC methods, these accessions were classified into three distinct groups. Containing most accessions, subgroup Ia includes all seeds, pod, and flower colours. Almost all accessions of subgroup Ib possessed cream-coloured, smooth and globose seeds. All cluster II accessions have a creeping habit. The CNRA collection being very diversified, it constitutes a useful resource for future cowpea improvement studies in Côte d'Ivoire.

Key words: Cowpea, accessions, genetic diversity, trait qualitative.

INTRODUCTION

The best selection strategies begin from the genetic diversity available in genetic resources. Techniques for assessing genetic diversity through phenotypic and genotypic variations are essential for improving the effectiveness of breeding programmes (Costa et al., 2017). Knowing trait variation can provide more complete understanding of the germplasm resources diversity among breeding materials (Guo et al., 2022). Indeed, assessment of genetic variability based on morphological markers is useful for early characterisation and selection in the field (Raina et al., 2020). Moreover, morphological data is also valuable for assessing and comparing patterns of diversity within and between populations (Veasey et al., 2008) as well as morphological and phenological performances are essential for high-potential agronomic result (Esan et al., 2023). A large

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number of studies (Egbadzor et al., 2014; Toyin, 2019; Barik et al., 2023) using phenotypic markers to understand cowpea (*Vigna unguiculata* (L.) Walp.) genetic diversity.

Native of Africa, cowpea is an important legume (Kouam et al., 2012) and where it constitutes the second most important crop, after groundnuts (*Arachis hypogea*) (Mateva et al., 2023). Among the world, Africa accounts for over 96% of total production, Nigeria, Niger and Burkina Faso being the three main producers (FAO, 2023). Cowpea has significant nutritional potential with high protein, carbohydrate and low fat content (Jayathilake et al., 2018). Its seeds, the most widely consumed, are used in a variety of food preparations. Fresh, young cowpea leaves are also used and ranked among the top four vegetables used in 24 African and seven Asian countries (Mohammed et al., 2021). It should be noted that the leaves (27 to 43%) contain a higher proportion of protein than the dried seeds (21 to 33%) (Anago et al., 2023). Cowpea also ensures the sustainability of production systems, thanks to its rapid soil cover. It protects the soil from erosion and weed invasion, and restores its fertility (Alemu et al., 2016; Das et al., 2018; Adu et al., 2021). It is also climate-resistant and helps to maintain sustainable agriculture. Cowpeas have multiple functions that help maintain good health (Frota et al., 2008; Dinore et al., 2022). Both wild and cultivated forms of this crop make it a well-diversified source conserved in many gene banks around the world: in Africa (International Institute of Tropical Agriculture (IITA), Nigeria), the United States (University of California, Riverside (UCR), State of California) and India (National Plant Genetic Resources Office (NBPGR), New Delhi).

In Côte d'Ivoire, known as the "haricot", cowpea is a highly prized crop for its seeds and leaves (Gore Bi et al., 2020). However, cowpea remains under-utilised crop and grown in rural areas on small-scale, low-income farmers and there are no national agricultural statistics on its yields. Moreover, very little information is available about the diversity of its genetic material. The various studies carried out are inadequate and do not allow us to explore its real potential. The local cowpea available today requires a good knowledge of all its characteristics. Information on the extent of genetic diversity will provide useful information for its improvement. Cowpea cultivation development could therefore contribute to food security and the Ivorian livelihoods improvement. Characterisation and evaluation of cowpea germplasm are essential for effective selection and conservation. Cowpea cultivation development could therefore contribute to food security and the Ivorian livelihoods improvement. Although quantitative traits enable the target traits exploration (Khan et al., 2022), qualitative traits affect the market value of genetic material (Adjimoti et al., 2017). These traits help at understanding plant intraspecific variations.

This study globally aimed at assessing genetic diversity of cowpea accessions grown in Côte d'Ivoire for their improvement in genetic programmes following qualitative traits. Specifically, this study aimed at assessing the Centre National de Recherche Agronomique (CNRA) cowpea accessions phenotypic diversity to determine the most discriminating qualitative traits and establish their genetic link. These results will provide a useful basis for selecting cowpeas.

MATERIALS AND METHODS

Plant

It consisted of *V. unguiculata* seeds of 135 accessions grown in Côte d'Ivoire. These accessions, from diverse agroecological zones, were provided by the CNRA.

Experimental site

Study was carried out on an experimental field in Bédiala (west-central Côte d'Ivoire), between latitude 7°09' 60.00" N and longitude 6°17' 60.00". Located in the Haut-Sassandra zone, this area enjoys a transitional humid tropical climate (Eblin et al., 2020). Its soils are ferrallitic and characterised by a thin humic horizon rich in organic matter, slightly acidic and well structured (Adjiri et al., 2019). Dry and wet seasons also alternate, with mean temperatures ranging from 24.65°C to 27.75°C (Konan et al., 2022). Bédiala is characterised by four seasons, two rainy seasons and two dry seasons (Tonessia et al., 2018). The average annual temperature is 25.6°C (Adjiri et al., 2019).

Experimental design

The field experiments were conducted over two years (2020 to 2021) using a randomised complete block design with two replications. Each replication consisted of 135 rows 4.5 m long, with 0.5 m spacing between rows. Each row contained 10 seed wholes each spaced by 0.5 m. Blocs were spaced by 2 m. The experimental plot total size was 73 m × 14 m, where contained 1350 plants, replicates were 1 m each spaced. Trials were regularly monitored during the growing season.

Traits measurement

Morphological characterisation was assessed following 15 qualitative traits selected from cowpea descriptor developed by the International Board for Plant Genetic Resources (IBPGR). The traits were recorded visually at different stages of plant growth in the field and after harvesting in the laboratory. The data were divided into three categories: (1) vegetative stage, (2) pod stage, and (3) seed stage (Table 1).

Statistical analysis

All the data collected from morphological traits of cowpea accessions were subjected to descriptive statistical analysis (phenotypic frequency distribution and Shannon-Weaver diversity index). Frequency distributions were used to calculate the Shannon-Weaver diversity index (H') for each trait in order to assess their diversity level according to the following formulae (Hennink and Zeven, 1990):

Table 1. Qualitative trait and phenotypic classes for 135-cowpea accessions evaluation.

Qualitative traits		Phenotypes
Vegetative stage	Terminal leaflet colour (Lco)	1: pale green, 2: intermediate, and 3: dark green
	Terminal leaflet shape (Lsh)	1: globose, 2: hastate, 3: oval, 4: rhomboid, and 5: sub-hastate
	Leaf texture (Pte)	1: membranous, 2: intermediate, and 3: coriaceous
	Growth habit (Gha)	1: climbing, 2: climbing erect, 3: creeping, 4: erect, 5: prostrate, 6: semi-erect, and 7: semi creeping
	Leaf hairiness (Pha)	1: glabrescent 2: short appressed hairs, 3: pubescent to hirsut
	Stem pigment (Spi)	1: pale green, 2: intermediate green, 3: dark green, 4: pale red, 5: red, 6: vtr (green with red trace)
	Flower colour (Fco)	1: cream, 2: dark purple, 3: mauve purple, 4: pale pink, 5: pink, 6: purple, 7: purple white, 8: white, 9: white purple, 10: white with purple spot
Pod stage	Pod colour (Pco)	1: brown, 2: chestnut brown, 3: chocolate, 4: khaki, 5: pale khaki, 6: pink, 7: pink khaki, 8: purple, 9: purple khaki, 10: yellow chocolate, 11: yellow khaki, 12: yellow purple
	Pod curvature (Pcu)	1: straight, 2: Slightly curved, 3: Curved, 4: arched
	Raceme position (Rpo)	1: Mostly above canopy, 2: In upper canopy, 3: Throughout canopy
Seed stage	Seed colour (Sco)	1: black, 2: brown argil, 3: brown olive, 4: brown orange, 5: brown red, 6: caramel, 7: cream, 8: orange, 9: red, 10: red-white, 11: terra brown, 12: white
	Seed eye colour (Eco)	1; absent, 2: black, 3: chestnut brown
	Seed hile colour (Hco)	1: brown, 2: chestnut brown, 3: grey, 4: orange, 5: yellow
	Seed texture (Ste)	1: smooth, 2: smooth to rough, 3: rough, 4: rough to wrinkled, 5: wrinkled
	Seed shape (Ssh)	1: globose, 2: ovoid, 3: crowder, 4: kidney, 5: rhomboid

$$H' = -\sum_{i=1}^{n} P_i \, Ln \, P_i / Ln(n)$$

where H'= Shannon-Weaver diversity index, Pi is the proportion of total entries number belonging to the class, n = genera number, and Pi= ni/N as the proportion of type I (ni = total number of individuals of the microbe in type i, N= total number of all individuals in type n).

Multiple correspondence analysis (MCA), carried out to determine the genetic variability level in the collection, detect differences between and within accessions and the most discriminating characters.

Based on the most discriminating characters from the MCA, a hierarchical cluster analysis (HCA) was assessed to establish relationships between accessions. Euclidean distances were estimated and a dendrogram was constructed using the ward D2 method. All analyses were performed using R software version 4.2.2.

RESULTS

Variability in morphological traits of 135 cowpea accessions

During plant growth, 15 qualitative traits were recorded at different stages (vegetative and maturity). Qualitative analysis revealed significant variation between accessions for all the traits analysed (Table 2).

Vegetative stage

At vegetative stage, leaf colour, shape, hairiness and texture, stem pigmentation, plant grown habit and flower colour were the studied traits.

The plant collection terminal leaflets colour ranged between pale green, intermediate green, and dark green. Most accessions (77) possess dark green leaves (57.04%), followed by 30 accessions with intermediate green leaves (22.22%) and 28 accessions carrying pale green leaves (20.74%). Leaf shape characteristics significantly varied among the 135 accessions. Thus, 65 accessions had rhomboid leaflets (48.15%), 32 accessions had sub-hastate leaflets (23.7%), 21 accessions with oval leaflets (15.56%), 13 accessions with hastate leaflets (9.63%), and only 4 accessions carrying globose leaflets (2.96%). This study shows that 48 accessions were characterized by cariceous-textured leaves (35.56%), 48 accessions by intermediate-textured leaves (35.56%) and only 39 accessions with membranous-textured

Table 2. Frequency distribution and Shannon-weaver diversity index for the 15 morphological traits of cowpea in collection.

Morphological description			Accessions frequency	Percentage contribution	Shannon's diversity index (H')
	Leaf Texture	Coriceous	48	35.56	1.00
		Intermediate	48	35.56	
		Membranous	39	28.89	
	Leaf shape	Globose	4	2.90	0.82
		Hastate	13	9.63	
		Oval	21	15.60	
		Rhomboid	65	48.15	
		Sub-hastate	32	23.70	
	Leaf colour	Dark green	77	57.04	0.89
		Intermediate green	30	22.22	
		Pale green	28	20.74	
	Stem pigment	Dark green	57	42.22	0.75
		Intermediate green	20	14.81	
		Pale green	41	30.37	
		Pale red	1	0.74	
		Red	2	1.48	
		Vtr	14	10.37	
Vegetative stage	Grown habit	Climbing	3	2.22	0.56
		Climbing erect	4	2.96	
		Creeping	37	27.41	
		Erect	82	60.74	
		Prostrate	1	0.74	
		Semi-creeping	5	3.70	
		Semi-erect	3	2.22	
	Flower colour	Cream	1	0.74	0.71
		Dark purple	20	14.81	
		Mauve pink	23	17.04	
		Pale pink	3	2.22	
		Pale purple	2	1.48	
		Pink	9	6.67	
		Purple	30	22.22	
		Purple white	1	0.74	
		White	40	29.63	
		White purple	4	2.96	
		White with purple spot	2	1.48	
	Plant hairiness	Glabrescent	34	25.19	0.99
		Pubescent to hirsut	49	36.30	
		Short appressed hairs	52	38.52	
Pod stage	Pod curtuve	Arched	12	8.89	0.89
		Curved	34	25.19	
		Slightly curved	64	47.41	
		Straight	25	18.52	

Table 2. Contd.

	Raceme position	In upper canopy	56		41.48	0.97
		Mostly above canopy		29	21.48	
		Throughout canopy		50	37.04	
	Pod colour	Brown		5	3.70	0.55
		Chestnut brown		3	2.22	
		Chocolate		1	0.74	
		Khaki		80	59.26	
		Pale khaki		1	0.74	
		Pink		1	0.74	
		Pink khaki		2	1.48	
		Purple		2	1.48	
		Purple khaki		1	0.74	
		Yellow chocolate		25	18.52	
		Yellow khaki		12	8.89	
		Yellow purple		2	1.48	
	Seed shape	Crowder		4	2.96	0.81
		Globose		30	22.22	
		Kidney		18	13.33	
		Ovoid		15	11.11	
		Rhomboid		68	50.37	
	Seed texture	Rough		6	4.44	0.73
		Smooth to rough		24	17.78	
		Rough to wrinkled		9	6.67	
		Smooth		81	60.00	
		Wrinkled		15	11.11	
	Eye colour	Absent		86	63.70	0.82
		Black		21	15.56	
		Chestnut brown		28	20.74	
Seed stage	Hile colour	Brown		9	6.67	0.70
		Chestnut brown		38	28.15	
		Grey		12	8.89	
		Orange		1	0.74	
		Yellow		75	55.56	
	Seed colour	Brown olive		12	8.89	0.86
		Brown orange		20	14.81	
		Black		1	0.74	
		Brown argile		5	3.70	
		Brown red		15	11.11	
		Caramel		4	2.96	
		Cream		27	20.00	
		Orange		4	2.96	
		Red		26	19.26	
		Red-white		1	0.74	
		Terra brown		2	1.48	
		White		18	13.33	
Overall mean						0.80

leaves (28.88%). About plant growth habit, most accessions (82) were erect (60.74%), 37 creeping (27.41%), 5 semi-creeping (3.7%), 4 climbing erect (2.96%), 3 semi erect (2.22%), 3 climbing erect (2.22%), and only one was prostrate (0.74%). Stem pigmentation expressed through six different colours: 57 accessions possess dark green stem (42.22%), 41 pale green (30.37%), 20 intermediate green (14.81%), 14 vtr (10.37%), 2 pink (1.48%) and one pale pink (0.74%). All the 135 accessions leaf hairiness expressed through three phenotypes: short and oppressed hairs for 52 accessions (38.52%), pubescent to hirsute hairs for 49 accessions (36.3%), and glabrescent for 34 accessions (25.19%). At blooming, the studied accessions produced flowers of different colours, mainly purple for 30 accessions (22.22%), followed by 20 accessions (14.81%) with dark purple flowers, 2 with pale purple (1.48%) and one producing purple white flower (0.74%). Nearly 40 accessions were white (29.63%), 23 accessions (17.04%) were mauve pink, 9 accessions (6.67%) were pink, 4 accessions (2.96%) were white purple, 3 accessions (2.22%) were pale pink, one accession (0.74%) were cream, and 2 accessions (1.48%) were white with purple spots.

Characteristics of cowpea pods and seed maturity

In cowpea, accessions maturity was accessed through pods and seeds characteristics. Pods maturity was characterized by their colour, curve, and raceme position. Seed shape, colour and texture, eye and hile colours characterized seed maturity.

Pod maturity characteristics

Pods racemes expressed through three phenotypes: on the canopy tip with 56 accessions (41.48%), throughout canopy with 50 accessions (37.04%), and above canopy with 29 accessions (21.48%). In terms of pod curvature, most accessions (64) carried slightly curved pods (47.41%) when some of them (34 accessions) disposed curved pods (25.19%), 25 accessions with straight pods (18.52%) and the other 12 accessions had arched pods (8.89%). There was a predominance of 79 accessions with khaki-coloured pods (58.52%), followed by chocolate yellow 25 (18.52%), khaki yellow 12 (8.89%), brown 5 (3.7%), chestnut brown 3 (2.22%), khaki pink 3 (2.22%), purple 2 (1.48%), purple yellow 2 (1.48%), one chocolate (0.74%), one pale khaki (0.74%), one pink (0.74%), and one khaki purple (0.74%).

Seed characteristics

About seed shape, most accessions (68) studied developed rhomboid seeds (50.37%), 30 containing globose seeds (22.22%), others carrying kidney seeds 18 (13.33%), ovoid seeds 15 (11.11%) and crowder seeds 4 (2.96%). Seed eye colour slightly variate among the studied accessions. More than 86 accessions (63.7%) had no seed eye colour, while 21 accessions (15.56%) were black and 28 accessions were chestnut-brown (20.74%).

Seeds hile colour trait showed that 75 accessions had a yellow colour, 37 were chestnut-brown, 13 grey, 9 brown and one orange. Globally, seeds appeared under 13 colours: cream for 27 accessions, red for 26, 20 brown orange, 18 white, 15 brown red, 12 brown olive, brown argil 5, 4 caramel, 4 orange, 2 terra brown, one black and one red-white. Seed texture varied from smooth to wrinkled. There were 81 accessions (60%) containing smooth seeds, followed by 24 smooths to rough (17.78%), 15 wrinkled (11.11%), 9 rough to wrinkled (6.67%), and 6 rough (4.44%).

Assessing the phenotypic diversity for each of 15 morphological trait, the Shannon's weaver diversity indices (H') varied from 0.55 to 1 with a mean value of 0.80. Among all the analysed traits, leaf texture expressed the higher (H'= 1), moderately high (0.70-0.89) for most of seed traits, while minimum for pod colour (H'= 0.55). The high index (H'= 0.80) indicated a high diversity level in the cowpea collection.

Multiple correspondence analysis (MCA) of cowpea morphological traits

Multiple correspondence analysis (MCA) of all the phenotypic traits showed that the first 3 components accounted for 19.17% of the total variability. From the 87 phenotypic classes identified, 26 derived from 12 traits contributing essentially to this axis formation. Accounting up to 10.74% of total phenotypic variation and defined by the 10 selected traits, axis 1 constituted the most important (Table 3). These traits were seed colour (white), pod colour (khaki, yellow chocolate), flower colour (white), eye colour (absent, chestnut brown), seed texture (rough to wrinkled, smooth, wrinkled), pod curvature (curved, slightly curved), raceme position (canopy tip, throughout canopy), grown habit (creeping, erect), plant hairiness (glabrescent, pubescent to hirsut), and stem pigment (pale green, vtr). Associated with four traits, seed colour (cream), seed shape (globose), raceme position (mostly above canopy), and growth habit (semi-creeping) axis 2 explained 4.45% of total variability. Defined by seed colors characters, colour (brown olive), eye (black) and hile (grey), axis 3 represented 3.98% of total variability. Figure 1 presents the 135 individual projections in plane 1-2. Based on axis 1, these accessions were classified into three groups.

Morphological cluster analysis

Based on the MCA 12 identified characters, the

Table 3. Matrix of Multiple correspondence analysis (MCA) explaining importance of phenotypic traits in squared cosine for the first three axis.

Variable	Axis 1	Axis 2	Axis 3
Variance	0.515	0.211	0.19
% of variance	10.723	4.404	3.958
Cumulative % of variance	10.723	15.127	19.085
Sco_ brown olive	0.04	0.01	**0.24**
Sco_cream	0.22	**0.35**	0.04
Sco_white	**0.43**	0.07	0.01
Pco_khaki	**0.23**	0.07	0.07
Pco_yellow chocolate	**0.60**	0.02	0.01
Fco_white	**0.58**	0.12	0.00
Ssh_globose	0.02	**0.40**	0.04
Eco_absent	**0.67**	0.08	0.07
Eco_black	0.17	0.00	**0.25**
Eco_chestnut brown	**0.36**	0.12	0.01
Hco_grey	0.04	0.01	**0.27**
Ste_rough to wrinkled	**0.21**	0.00	0.03
Ste_smooth	**0.26**	0.17	0.00
Ste_wrinkled	**0.31**	0.02	0.03
Pcu_curved	**0.79**	0.03	0.00
Pcu_slightly curved	**0.25**	0.00	0.20
Rp_in upper canopy	**0.35**	0.04	0.01
Rp_mostly above canopy	0.03	**0.24**	0.00
Rp_throughout canopy	**0.55**	0.05	0.01
Gha_creeping	**0.83**	0.04	0.00
Gha_erect	**0.55**	0.02	0.00
Gha_semi-creeping	0.00	**0.34**	0.00
Pha_glabrescent	**0.62**	0.05	0.01
Pha_pubescent to hirsut	**0.22**	0.01	0.00
Spi_pale green	**0.24**	0.05	0.09
Spi_vtr	**0.34**	0.02	0.02

hierarchical component analysis (HCA) was carried out. Based on Euclidean distances, dendrogram (Figure 2) allowed classification of 135 accessions from the CNRA cowpea collection into two main clusters. Cluster I was subdivided into two subgroups: Ia (red) and Ib (green) containing, respectively 97 and 10 accessions, while subgroup Ib was composed of accessions. Cluster II (purple) comprised 28 accessions.

Containing most accessions, subgroup Ia includes all seed, pod and flower colours. About 92.78% of the accessions are hairy, while over 68.04% have smooth seeds. Moreover, seed forms were 54.64% of accessions for rhomboid form, 7.22% for kidney, 15.46% for ovoid seeds, 19.59% for globose and 3.09% for crowder seeds. Over 80.41% of accessions were coriceous and 78.35%, no coloured eye. Raceme position varied: 57.73% accessions had pods on canopy tip, 21.65% above canopy and 20.62% throughout the canopy. Most accessions, 59.79%, produced slightly curved pods, 20.62% straight, 11.34% arched, and 8.25% curved. Within the group, 41.24% of accessions had pale green, 40.21% dark green, and 18.56%, intermediate green stem.

The subgroup Ib consisted of khaki-coloured pods, white flowers and smooth seeds on hairiness plants. From 10 accessions, 9 (90%) had globose, cream-coloured seeds with brown eyes and hile. Futhermore, 70% carried pods mainly on canopy tip and dark green leaves, whereas 50% were semi-creeping with straight and slightly curved pods.

Cluster II contained creeping accessions with curved pods. About 92% of the accessions were hairless while most (96%) had them in canopy. In addition, 60% of the accessions had chocolate-yellow pods. From these accessions, 50% produced rhomboid seeds, 39.29% kidney and 10.71% globose seeds. On the other hand, 50% produced white seeds, 46.43% cream and 3.57% caramel ones. Seed eye colour was mainly yellow for 78% of the accessions while only one accession produced uncoloured eye. In this group, 50% of

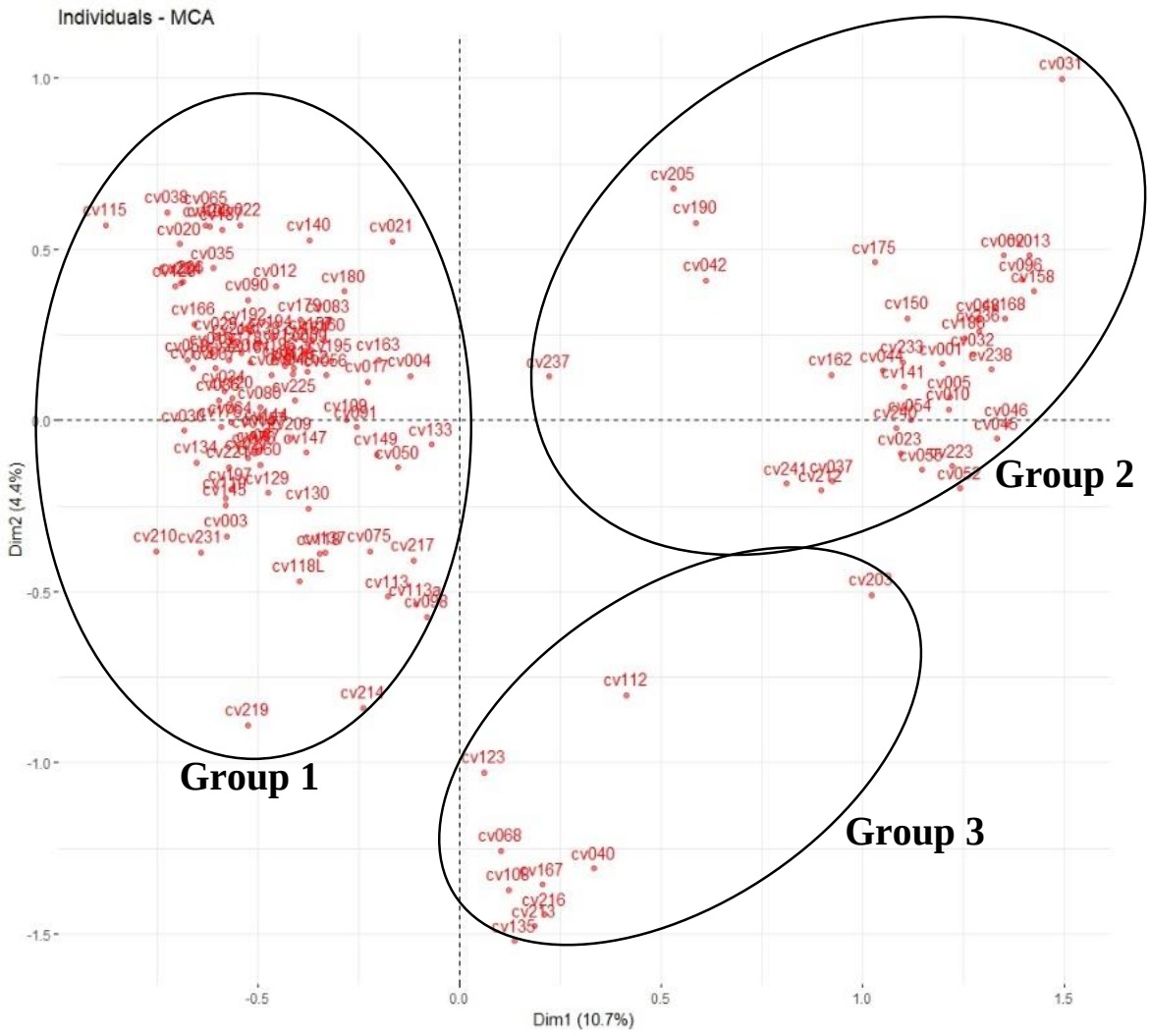

Figure 1. Factorial representation of the 135 accessions analysed from the CNRA cowpea collection.

accessions had vtr-coloured stem pigments, compared with 39.29% dark green and 10.71% red.

DISCUSSION

Genetic diversity is the basis for successful crop improvement and can be assessed using a variety of methods (Fufa et al., 2005). Germplasm phenotypic evaluation enables genetic material characterisation and conservation. This method was the way followed to explore an eventual genetic variability of Côte d'Ivoire cowpea germplasm at different stages of plant development. The present study revealed a high level of genetic diversity for the 135 accessions studied. Phenotypic classes corresponding to the 15 qualitative traits ranged from 3 to 12. This result indicates a high level of phenotypic variability, suggesting presence of genetically distinct accessions of this collection. Barik et al. (2023) already reported significant variation among all the 10 qualitative traits they studied. Moreover, relative frequencies of each trait phenotypic characteristics showed they were irregularly distributed, with predominance of certain phenotypic characteristics. This predominance could indicate the presence of dominant phenotypes. According to Terzić et al. (2020), qualitative traits play an important role in cultivar development process, depending on the desired phenotype as the ultimate breeding goal. Allelic richness, measured by the Shannon-Weaver diversity index, showed variability in each studied trait. Among 15 qualitative traits examined, leaf texture (H'= 1.00) and plant hairiness (H'= 0.99) were the most variable, while pod colour (H'= 0.55) and growth habit (H'= 0.56) showed the least variation. High variability was provided by cowpea leaf texture and accessions were classified into 3 categories, groups 1

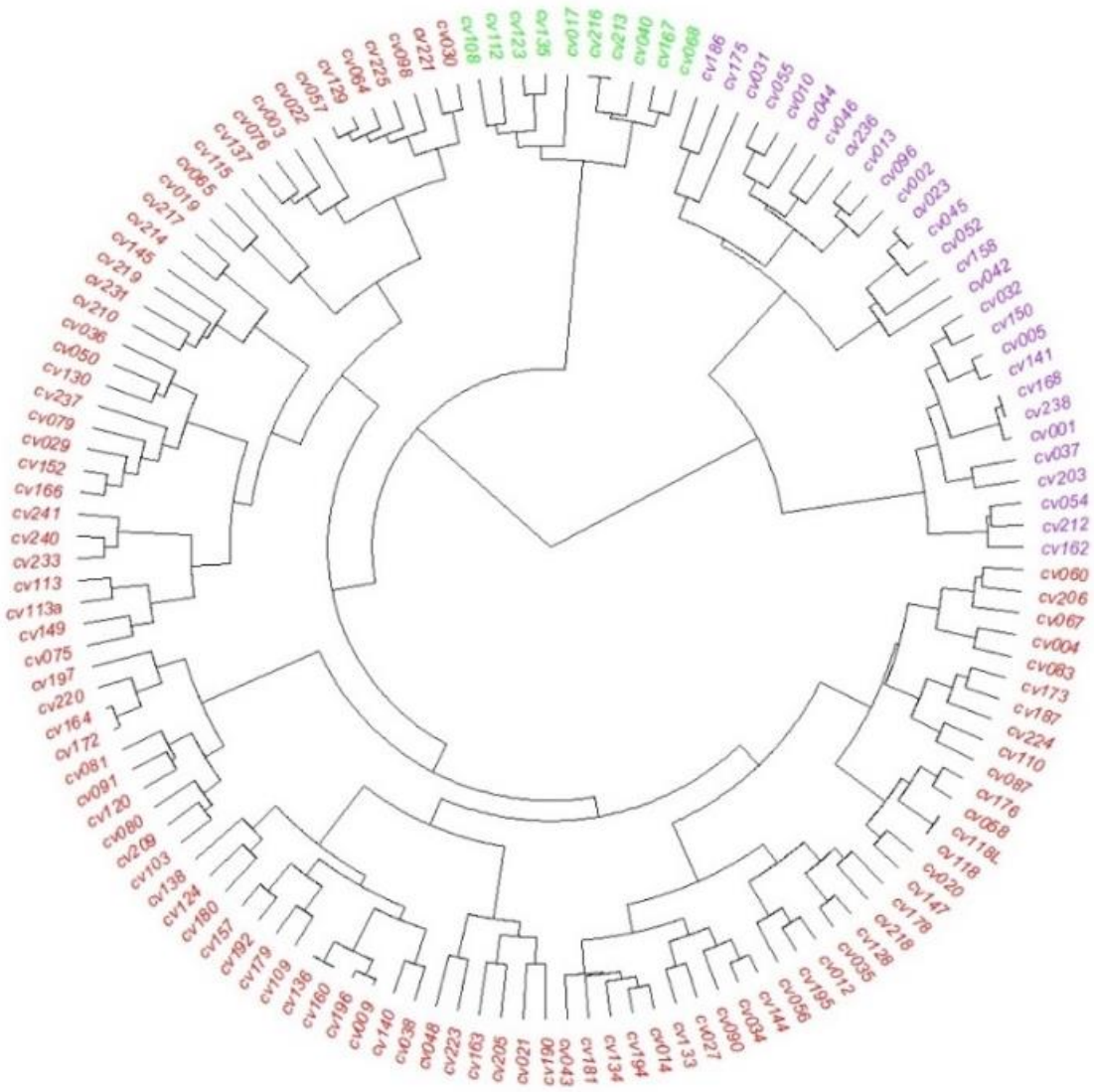

Figure 2. Dendrogram on the morphological variability of the qualitative characteristics of 135 cowpea accessions in the collection based on the Ward D2 classification method using Euclidean distance.

and 2 consisted of the same accession's numbers, respectively coriaceous (35.56%) and intermediate (35.56%) textures. Group 3 had membranous texture (28.89%). Plant hairiness allowed classification of the cowpea accessions into three groups. Gathering 38.52% of accessions, the first group seeds were characterized by short oppressed hairs, the second (36.3%) by pubescent to hirsute hairs and the third (25.19%) by glabrescent ones. Toyin (2019) reported similar results which allow them cowpea genotypes classification into three groups and differs from those noted by Barik et al. (2023).

Multiple correspondence analysis (MCA) was assessed to understand each studied trait contribution to phenotypic variation among accessions and identify the most discriminating ones. According to Doumbia et al. (2013), this step is fundamental to any selection and conservation programme. Indeed, our three first axes explained most total phenotypic variance (19.17%). With a 10.74% variance, axis 1 was correlated with 10 of 15 studied traits. Traits concerned colours of seed, pod, flower and eye; seed texture, pod curvature, raceme position, grown habit, plant hairiness, and stem pigment. Therefore, seed colour (Ishikawa et al., 2020) and both colour and texture (Lopes et al., 2003) being taken into account, respectively in farmers and traders selection criteria, this axis can be retained for possible selection studies. So, this analysis allowed the 135 accessions classification into very distinct three groups.

Based on discriminating quantitative traits, hierarchical classification allowed the 135 accessions sharing into three groups. This suggests that the accessions of CNRA

cowpea collection were greatly diversified among them. This result confirms those obtained with the MCA, which revealed three phenotypic groups. Covering all the seeds, pods and plants characteristics, group 1a is very diversified. As such, it could be conserved, and then used as core collection.

Conclusion

This study revealed high level of genotypic variation in the CNRA cowpea collection. The MCA and HAC analyses lead to similar results, allowing the 135 accessions classification into three genetic groups. Each group characteristics exploration could provide more genetic diversity for breeding programmes. Seed colour was on all the three factorial axes, indicating its importance for cowpea breeding. These results provide guidelines for possible cowpea improvement in Côte d'Ivoire.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Purification of stilbene synthase from *Phaseolus vulgaris* and determination of its catalytic activity

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Stilbene compounds, secondary metabolites produced by plants in response to external environmental stimuli, are garnering increasing attention. Stilbene synthase (STS) plays a crucial role in their synthesis. This study focuses on the purification of stilbene synthase from *Phaseolus vulgaris* and the determination of its catalytic activity. The stilbene synthase gene obtained from the Phaseolus genome was expressed in large quantities by constructing engineered strains of *Escherichia coli*. Furthermore, the active proteins produced by the stilbene synthase gene in *Phaseolus* plants were measured by assessing the activity of stilbene synthase obtained from both His column and Q column purifications. In this study, the active stilbene synthase gene was initially identified from the Papilionoideae. Exploring stilbene synthase contributes to a deeper understanding of the relationship between plant stress resistance and plant evolution.

Key words: Stilbene synthase, *Phaseolus vulgaris*, His column, Q column, PvSTS.

INTRODUCTION

The phenyl propionic acid metabolic pathway, a distinctive metabolic route, serves as a common mechanism for generating secondary metabolic products in most plant species. Its primary goal is to produce flavonoid glycosides, anthocyanins and various polymeric lignin substances. Flavonoids assist plants in resisting UV damage, certain flavonoid glycosides and isoflavone glycosides act as inducers to promote the expression of nodulation genes in rhizobia bacteria, anthocyanins participate in the synthesis of plant pigments, and poly lignin provides structural support and functions as a range of plant antitoxins (Martínez-Márquez et al., 2016).

These compounds exhibit diverse pharmacological and biological activities. Currently, 23 families and 59 genera of plants (Table 1) are known to produce stilbene compounds (Pecyna et al., 2020). In the terpenoid metabolic pathway of plants, specifically the phenyl propionic acid pathway, stilbene synthase plays a crucial role in synthesizing stilbene derivatives from coenzyme A intermediates (such as p-coumaroyl CoA, cinnamyl CoA, etc.) and malonyl CoA as reactants. Thus, this enzyme is pivotal in the synthesis of stilbene compounds (Abe and

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Table 1. Distribution table of stilbene compounds in plant kingdom.

Number	Class	Family
1	Leguminoese	Arachis, Cassia, Sophora, Trifolium, Bauhinia, Ilex, Dalbergia, Pterolobium, Derris, Amorpha, Pterocarpus, Cajanus, Guibourtia, Laburnum, Pericopsis, Schotia, Haplormosia, Youacapoua, Centrolobium
2	Moraceae	Morus, Artocarpus, Cudrania, Chlorophora
3	Dipterocarpaceae	Hopea, Shorea, Balanocarpus
4	Vitacese	Vitis, Parthenocissus, Ampelopsis, Cissus, Rhoicissus
5	Pinaceae	Pinus, Picea
6	Polygonsceae	Polygonum, Rheum
7	Mytaceac	Eucalyprus, Angophora
8	Combretaceae	Combretum
9	Myristicaceae	Knema, Virola
10	Cyperaceae	Carex, Scirpus
11	Lamiaceme	Scutellaria, Salvia, Vitex
12	Anacardiaceae	Rhus
13	Saxifragacesc	Hydrangea
14	Phytolaccaceae	Petiveria
15	Liliaceac	Veratrum, Smilax
16	Fagaceae	Nothofagus
17	Betulaceae	Alnus
18	Urticaceae	Toxylon
19	Umbelliferae	Pleuraspermum, Ferula
20	Cupressaceae	Juniperus
21	Gnetaceae	Gnetum
22	Ericaceae	Gaylussacia
23	Poaceae	Festuca

Morita, 2010). Stilbene compounds, often synthesized in response to plant stress, are secondary metabolites produced by plants when exposed to external environmental stimuli and belong to the terpenoid class (Hanhineva et al., 2009). As research progresses, there is increasing attention on these bioactive compounds, and elucidating the key enzyme in stilbene compound synthesis, namely stilbene synthase, holds great significance.

The primary route for generating stilbene compounds is through the phenyl propionic acid metabolic pathway (Abe and Morita, 2010). In the synthesis pathway of stilbene compounds, three enzymes play a crucial role in transforming related organic matter from primary metabolites. Phenylalanine is first converted to Coenzyme A (CoA)-activated thionyl phenylpropionate. This process involves the deamination of phenylalanine by phenylalanine lyase (PAL) to form cinnamic acid. Subsequently, cinnamic acid undergoes catalysis by cinnamic acid 4-hydroxylase (C4H) to produce p-coumaric acid. Under the action of p-coumaric acid CoA ligase (4CL), a thioester bond is formed at the carboxyl group of p-coumaric acid, resulting in the production of p-coumaroyl CoA (Yu et al., 2005). The next step involves the catalysis of p-coumaroyl CoA and two malonic monoacyl-CoA by stilbene synthase (STS), leading to the synthesis of secondary metabolites known as stilbenes (Figure 1). Additionally, p-coumaroyl CoA and other compounds can enter the flavonoid and isoflavone synthesis pathway under the catalysis of chalcone synthase (CHS).

Stilbene synthase is typically produced with stilbene compounds, but it has now been discovered that there may also be an enzyme similar to STS (PvSTS) in *Phaseolus vulgaris*. This plant is known to contain no stilbene compounds, so it is generally believed that this plant does not possess the physiological activity of stilbene synthase (Rosano and Ceccarelli, 2014). However, a genetic comparison has identified a protein in the plant's genome with a sequence similar to that of ordinary stilbene synthase. It is now necessary to prove whether the protein exhibits the activity of ordinary stilbene synthase.

MATERIALS AND METHODS

Construction of recombinant plasmid and expression of target protein

The first step is to construct a gene expression vector using Gibson

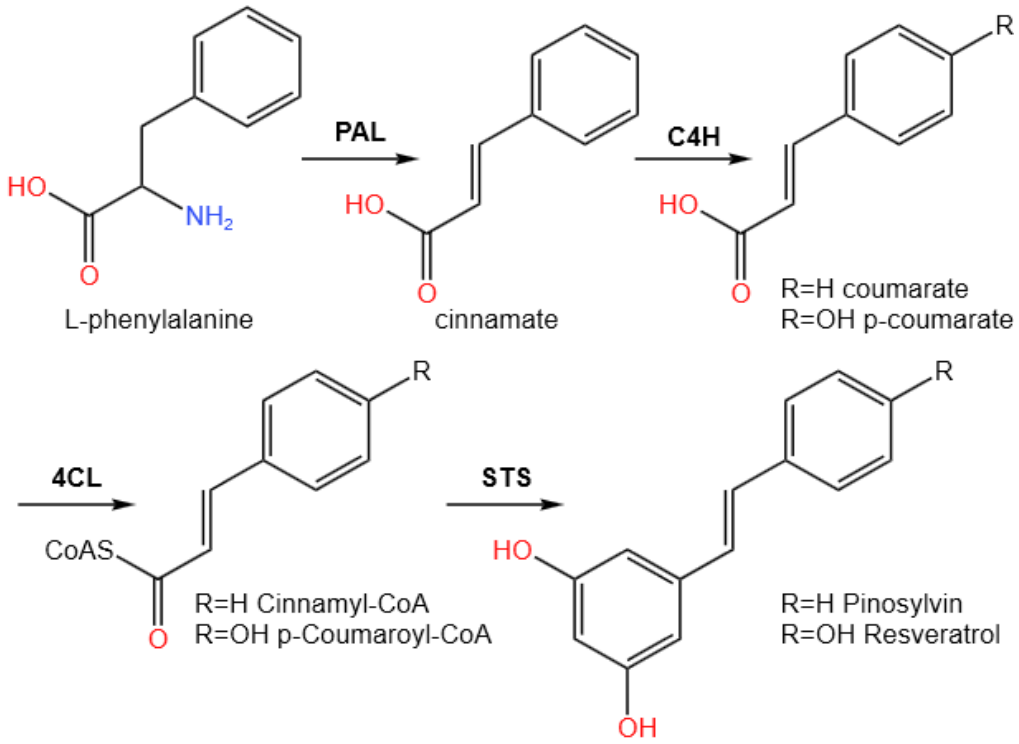

Figure 1. The metabolic process of stilbene compounds.

assembly (Figure 9a). The target gene was amplified by high-fidelity PCR with specific primers to obtain the target gene sequence containing homologous sequences with the plasmid vector. The obtained genes and plasmids were mixed and cultured in Gibson assembly master mix for homologous recombination (Figure 9b). The resulting recombinant plasmids were separated by electrophoresis, and the purified plasmid was obtained. After confirming the correct molecular weight through enzyme digestion electrophoresis, DNA sequencing was performed to confirm the successful construction of the recombinant plasmid (Beccaria and Cabooter, 2020).

The recombinant gene expression vector can be transformed into *E. coli* cells using the receptor transformation technique. The transformed *E. coli* cells were plated on a medium containing antibiotic, and the recombinant cells that successfully expressed the target gene were selected. Following this, single colony isolation was performed on the medium, and the purified strains isolated were tested through cleavage and PCR. The success of the transformation could be determined after electrophoresis (Hanhineva et al., 2009). The successfully transformed strains were selected for culture, and after reaching a certain extent of growth, IPTG was added to induce protein expression.

Protein extraction and purification

After a period of cultivation, the culture medium was centrifuged, and the thalli were separated. Buffer was added and suspended, followed by ultrasonic crushing and filtration. The resulting protein solution was then separated and purified using a His column (Gaberc-Porekar and Menart, 2005). The presence of proteins was assessed through SDS-page electrophoresis (Figure 2). Subsequently, the protein solution obtained underwent treatment with osmosis and desalting before being separated and repurified using a Q-column. The location and purity of the proteins were determined by electrophoresis (Marbach and Bettenbrock, 2012) (Figure 3). Once the highly purified protein was obtained, it was concentrated and stored at a low temperature (Figure 4).

Determination of protein activity and LC-MS

The assay, involving an activity test with the obtained enzymes, demonstrated that PvSTS exhibits the protein activity typical of common STS. Previous research has outlined two reactions catalyzed by STS enzymes:

Reaction 1: p-Coumaroyl CoA and malonyl CoA were utilized as substrates, yielding Resveratrol as the main product and Naringenin chalcone as the by-product (Figure 10). Naringenin is formed spontaneously due to the unstable molecule of Naringenin chalcone.
Reaction 2: Caffeoyl CoA and malonyl CoA were used as substrates, resulting in Piceatannol as the primary product and Eriodictyol as the by-product (Figure 11).

Experiments were designed based on these principles to confirm whether PvSTS possesses the catalytic activity of STS. P-coumaryl CoA served as the foundational substrate, excess malonyl CoA was added as an excipient, and 10 μg of purified PvSTS was included for heat preservation. Following 24 h of incubation, the solution underwent filtration and separation to eliminate proteins and other macromolecular impurities. LC-MS detection was conducted. The samples were replicated three times, and the blank control group was maintained (Caruso et al., 2011). The spectral line corresponding to the product was measured and analyzed to determine the generation of the corresponding product, thereby assessing sample activity (Figures 5 to 8).

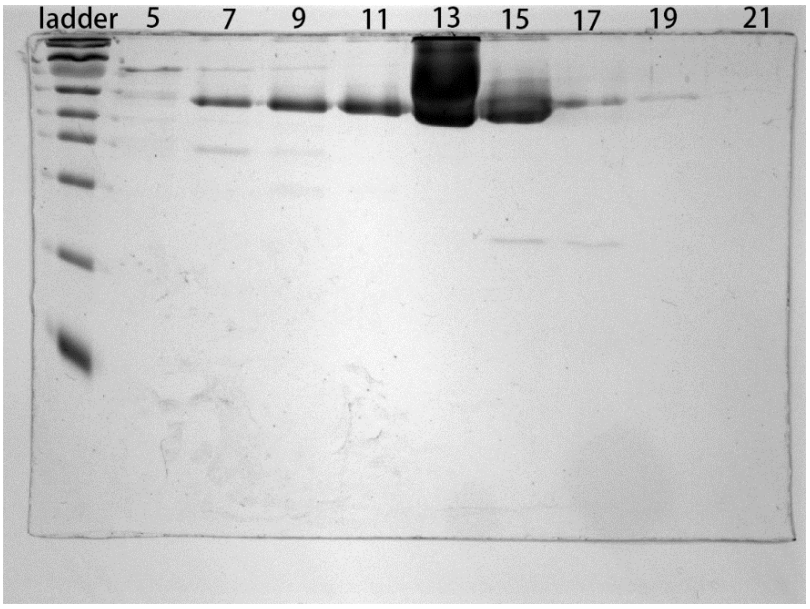

Figure 2. Electrophoretic results after His column.

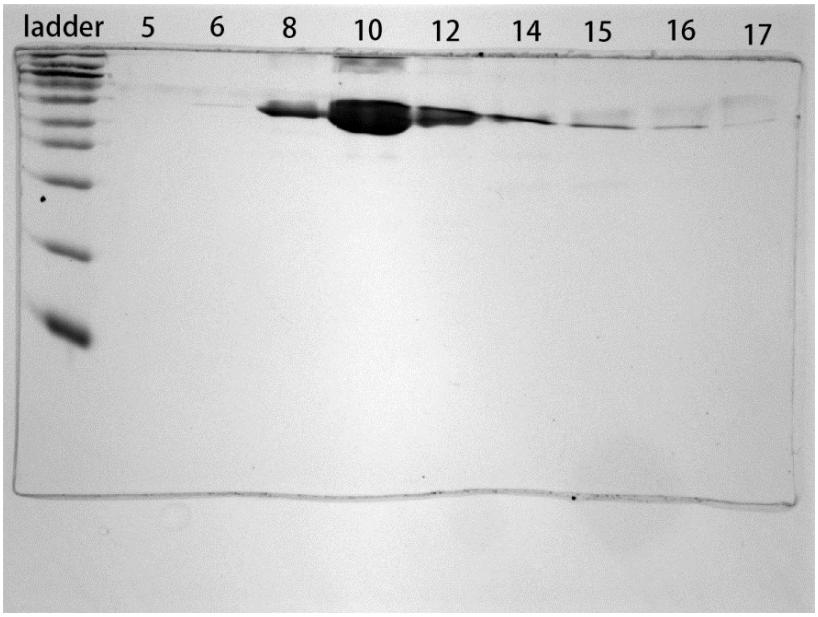

Figure 3. The result of electrophoresis after Q column.

RESULTS AND DISCUSSION

During the purification process, it is essential to assess the protein content and purity through electrophoresis. In these experiments, a series of images (Figures 2 to 4) were obtained to validate the presence of the protein.

The results indicate that the purified protein exhibits high purity with minimal impurities, making it suitable for protein activity detection (Figures 5 to 8). Subsequent analysis revealed the presence of the expected products in sufficient quantities, confirming that PvSTS possesses the general catalytic activity characteristic of STS proteins.

In conclusion, the experiments demonstrate that PvSTS exhibits the catalytic activity typical of common STS proteins.

The objective of this experiment was to investigate whether PvSTS exhibits the catalytic activity typical of

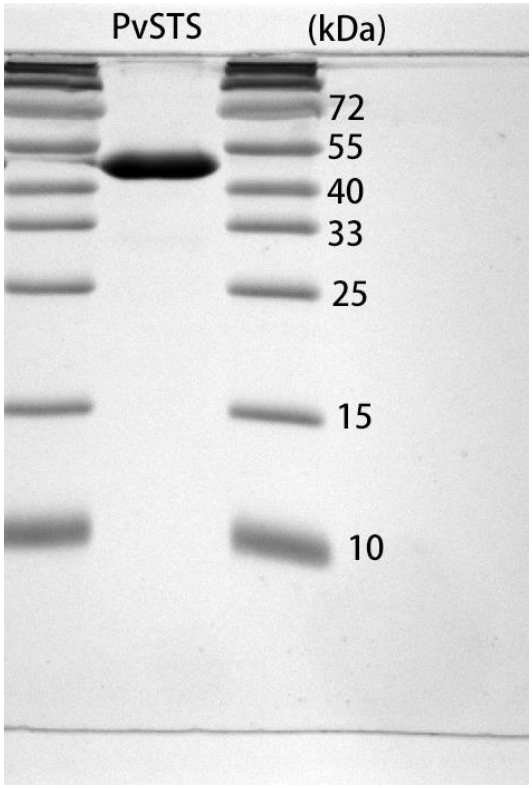

Figure 4. The result of electrophoresis after purification.

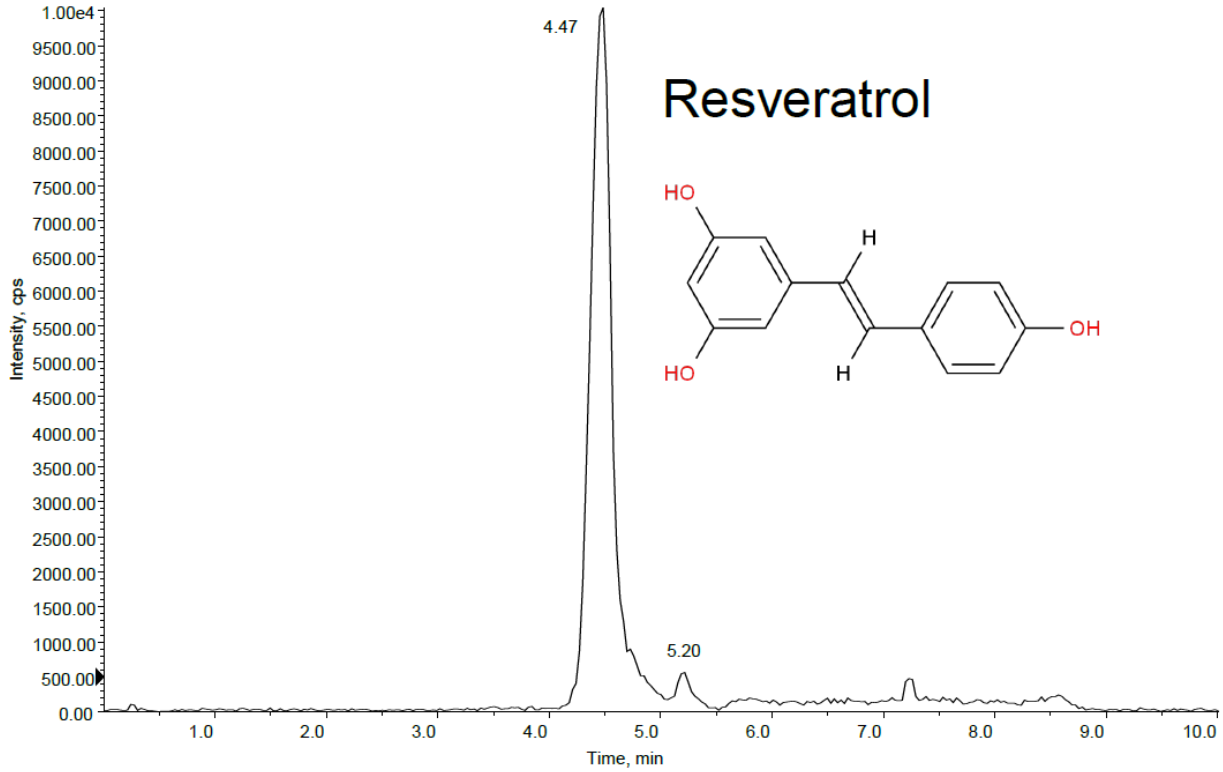

Figure 5. The result of resveratrol of LC-MS after reaction 1.

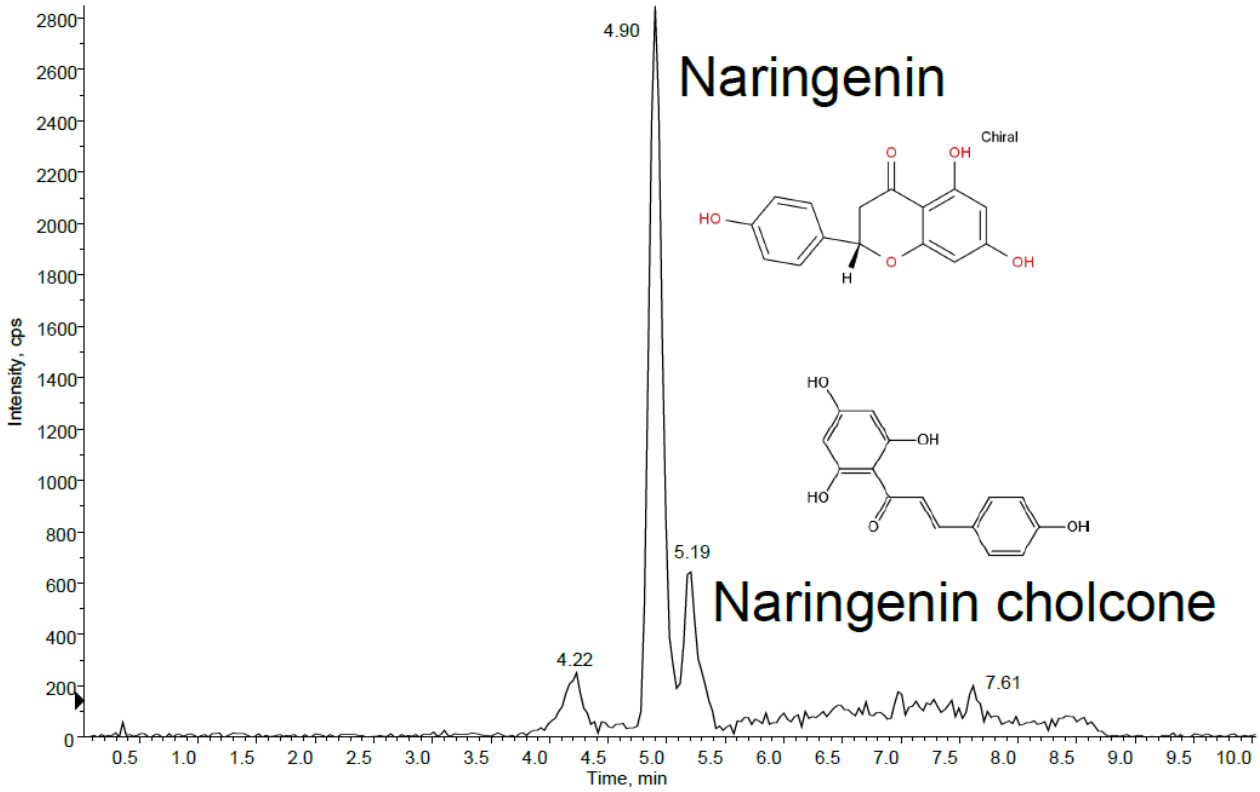

Figure 6. The result of naringenin of LC-MS after reaction 1.

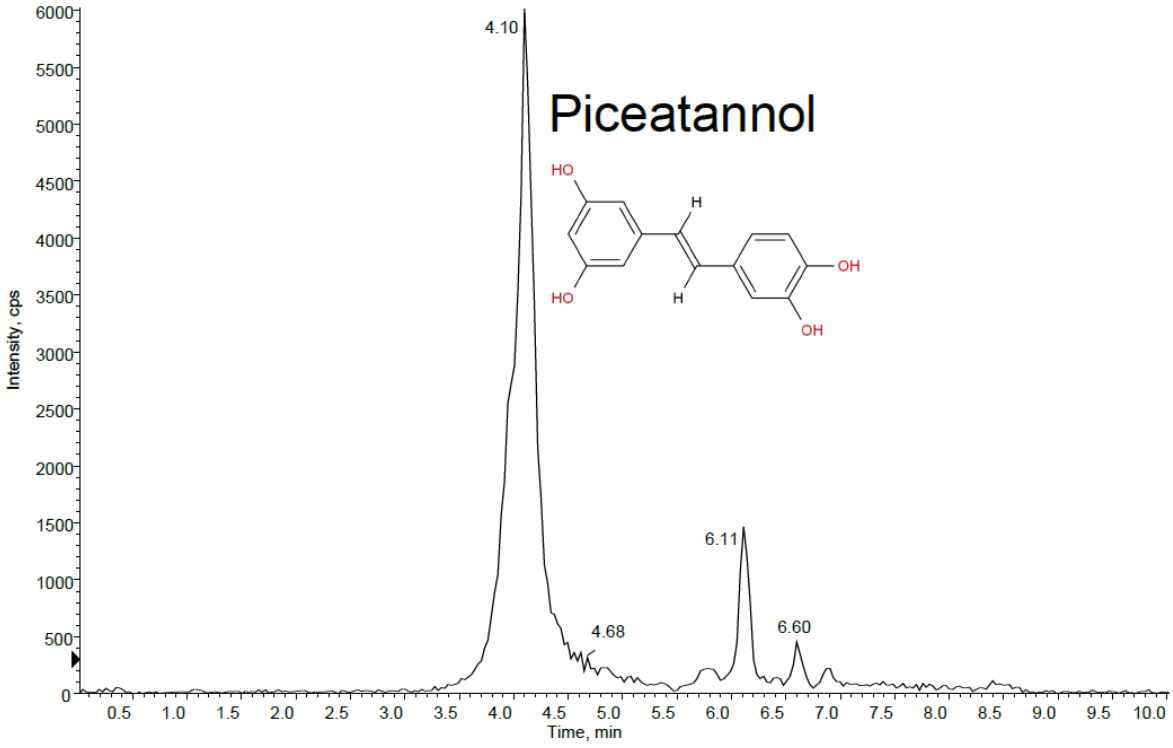

Figure 7. The result of piceatannol of LC-MS after reaction 2.

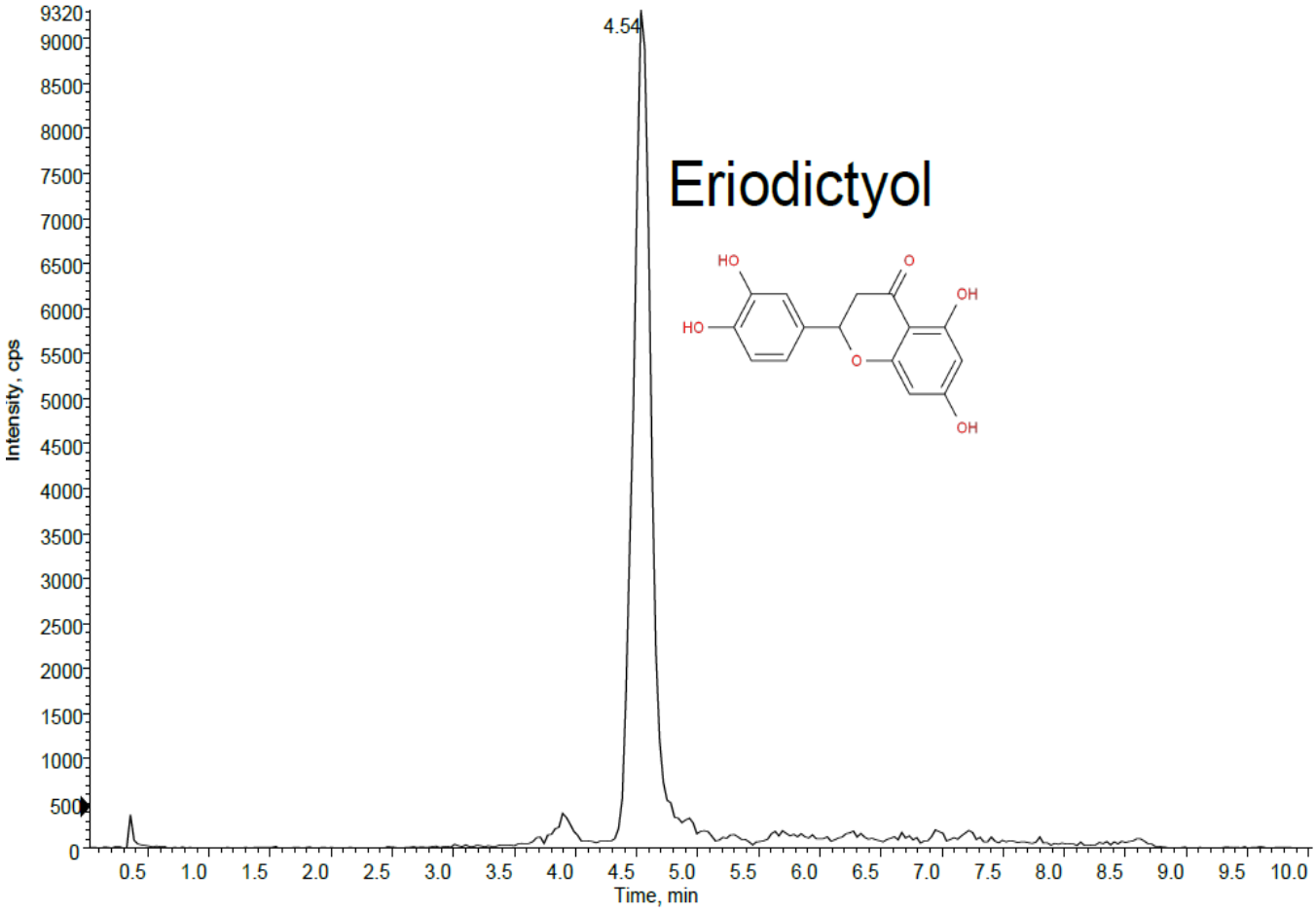

Figure 8. The result of eriodictyol of LC-MS after reaction 2.

ordinary stilbene synthases. However, as the bean plant itself could not naturally produce this protein, it necessitated genetic engineering for expression and extraction. The study is organized into three main phases.

1. Protein expression: The gene sequence encoding the protein was isolated and then recombined with the gene expression vector of engineered bacteria to construct a recombinant plasmid. Subsequently, the recombinant plasmid was introduced into the engineered bacteria for cultivation and expression (Spriestersbach et al., 2015).
2. The engineered bacteria underwent crushing and centrifugation to extract proteins for separation. The extracted proteins were then purified using a His column and a Q column, resulting in protein samples with sufficient purity for activity determination (Lu et al., 2008).
3. The protein was mixed with the corresponding substrate for the reaction. After a specified duration, the presence of the expected product was determined using LC-MS (Li and Elledge, 2012).

Although no derivatives of STS have been found in *P. vulgaris*, this study suggests that the bean still possesses the enzyme needed to produce the compound. This observation may indicate that this enzyme is common in plants and plays an irreplaceable role in physiological processes beyond the synthesis of stilbene compounds (Marbach and Bettenbrock, 2012). As a crucial compound in plant stress response, stilbene derivatives also underscore the significance of STS series proteins in related reactions.

The discovery of PvSTS with general stilbene synthase bioactivity in *P. vulgaris* demonstrates that the absence of stilbene compounds does not equate to the absence of stilbene synthase. This suggests that the protein, which remains biologically active in plants, may be involved in other vital life processes (Zhao et al., 2010).

The consistency in the function and sequence of stilbene synthase across different plants also indicates a degree of conservation. This suggests that this protein may play more crucial and essential physiological roles in plants beyond the synthesis of stilbene compounds.

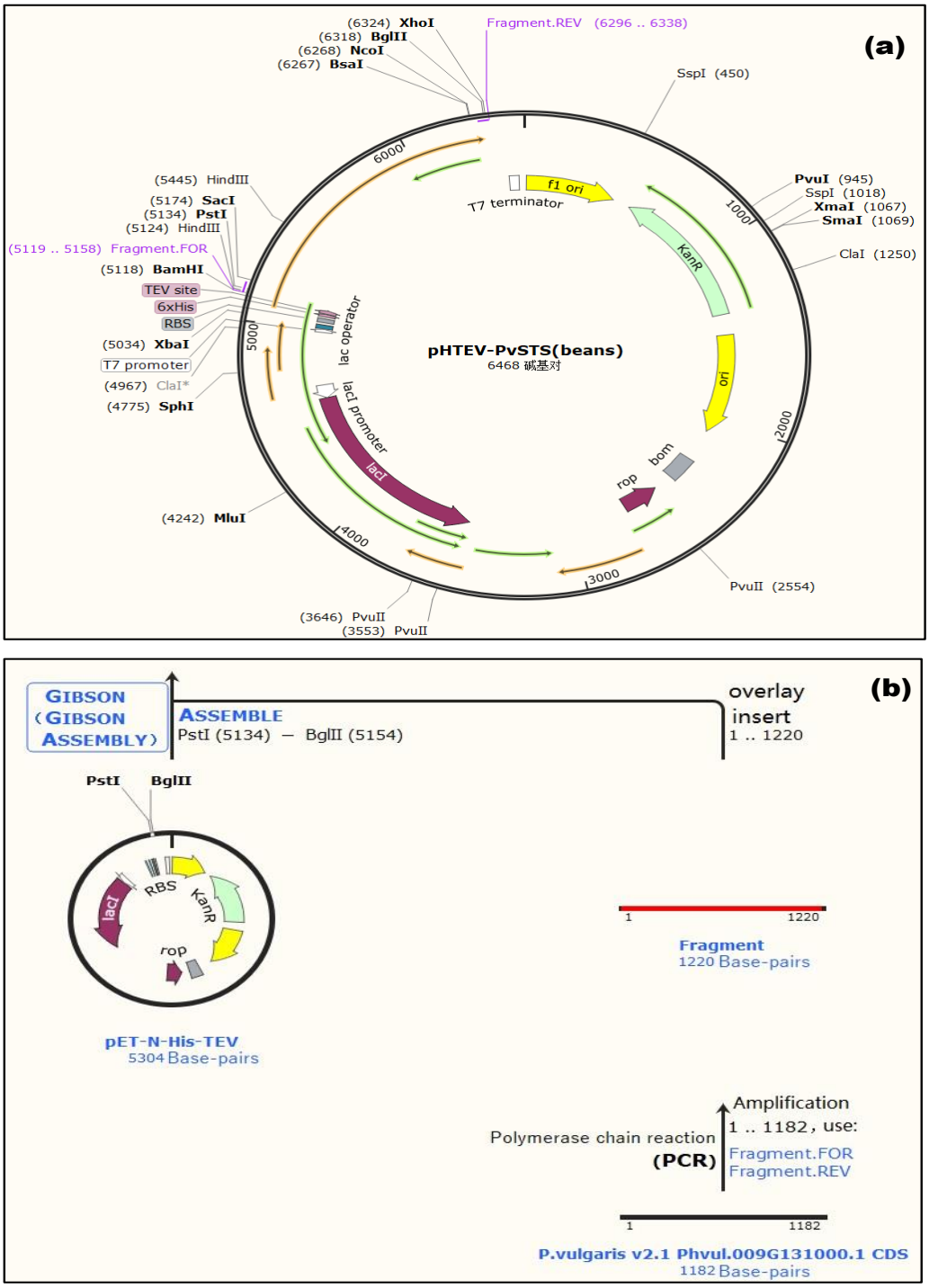

Figure 9. The principle of construction of recombinant plasmid. (a) Constructing a gene expression vector with Gibson assembly; (b) Homologous recombination.

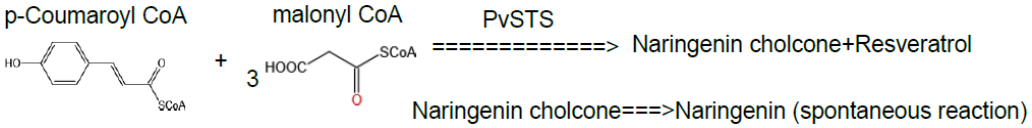

Figure 10. The reaction of p-Coumaroyl CoA and malonyl CoA.

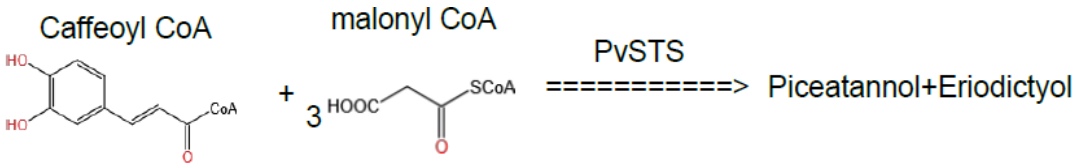

Figure 11. The reaction of caffeoyl CoA and malonyl CoA.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

THE DATA AVAILABILITY STATEMENT

All data, models, and code generated or used during the study appear in the submitted article.

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Estimates of genetic variability in ultraviolet irradiated populations of summer squash

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Summer squash is an important member of the family Cucurbitaceae. It is one of the most popular vegetable crops cultivated in Egypt. Increasing yield and improving fruits quality are the targets for growers. Therefore, this study aimed to evaluate the extent of genetic variations, heritability, as well as expected genetic advance for growth and yield related traits in four genotypes of summer squash subjected to three doses of ultraviolet irradiation. Data were subjected to the analysis of variance for the important components of yield and agronomic traits. The results showed relatively small differences between phenotypic and genotypic variance for the number of leaves per plant, chlorophyll concentrations in leaves, and fruits. Larger magnitude of difference was obtained between phenotypic and genotypic coefficient of variations in the expression of leaf area, female flowers, male flowers developed per plant, and carotenoids concentration in fruits, indicating that environmental factors played predominance role in the expression of these traits. Moderate heritability value was obtained for fruits weight per plant coupled with high genetic advance as a percentage of mean. Therefore, selection based on the phenotypes will improve the mean performance of fruits weight per plant in the selected progenies.

Key words: Summer squash, ultraviolet irradiation, genetic components, genetic advance, heritability.

INTRODUCTION

Summer squash is the edible immature fruit which belongs to the economically important family Cucurbitaceae. Summer squash, *Cucurbita pepo* L. generally displays more male flowers and few female flowers. This leads to lowering its fruit yield. The sex expression of *C. pepo* L. is resolved by genetics and ambience as photoperiods, temperature, etc. This crop was cultivated in Egypt for local consumption, as well as for foreign exporting market. It contains a huge amount of vitamins A, C, E, B6, niacin, thiamin, and minerals (Bose et al., 2000). Increasing squash yield and fruit quality are the main targets for breeders. *C. pepo* L. is native to North America and the wild types can be found in northeastern Mexico and central USA (Paris, 2008). Kooistra (1967) found a clear effect of temperature and day length on cucurbits sex expression. Furthermore, long days and high night temperatures induced shift towards increased male flowers and decreased female flowers. Whereas, low temperatures and short photoperiod increased female flowers and decreased male flowers. This may affect pollination and fruit setting. This will cause decrease of its fruit yield in summer

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season compared to the early spring season. *C. pepo* L. is a cross pollinated vegetable crop having a diploid number of chromosomes (2n = 2X = 40) which is cultivated for its fruits to be used in human nutrition (Jasim and Esho, 2021). The sexual development in summer squash is monoecious, occurring in separate locations on the same plant, as well as isolated in the leaf axils. Furthermore, female flowers are usually less numerous than male flowers perhaps they have an impact on the carrying of elongated ovary (Filgueira, 2008).

Induced mutations in *C. pepo* via UV irradiation have been used to generate genetic variability which successfully utilized to improve yield and its components in summer squash. Mutagenesis is a potential tool to be employed for summer squash improvement (Khan et al., 2006). To create high-yielding genotype in summer squash requires the existing genetic variations for yield and its components. The phenotypic variability observed included genetic and environmental causes. The estimates of heritability alone do not indicate a knowledge about the expected genetic gain in the next generation, but have need to estimates of genetic advance, the change in mean value between generations (Wani and Khan, 2006). The relative performance of different genotypes differed in different environments because of genotype by environment interaction. The reliability of genotype expression across different environmental conditions was an important consideration in plant breeding program. A successfully developed new genotypes may have a stable genetic expression and broad adaptation over a wide range of environmental conditions, as well as to high yielding potential (Azad et al., 2009).

Summer squash prevails high degree of genetic variability due to the presence of high percentage of out crossing (Hanelt and Mettin, 1989). A large genetic variability identified in summer squash including floral biology, fruit size, fruit colour, fruit composition, tolerance to biotic and abiotic stresses, and yield potential efficiency (Duc et al., 2010). It is obvious that genetic gain from selection depending on the genetic variations and the magnitude of the heritable protein of these genetic variations. Thus, the information about the degree of genetic variations exist in summer squash is critical to design a suitable breeding program (Fikreselassie and Seboka, 2012).

Variability is the presence of genetic variations among individuals due to the differences in their genetic background and/or the environment in which they live (Falconer and Mackay, 1996). Effective selection needs information about the nature and magnitude of genetic variations across populations (Yagdi, 2009). The selection of promising genotypes from large genetic base in the population to be subjected with a sequence utilization for hybridization is a good tool for improving the productivity of summer squash (Mulugeta et al., 2013). The highest production of summer squash was referred to Turkey, Italy, Egypt, Spain, USA, and Mexico (Paris, 2008). It is so considered that Egypt is the most famous *C. pepo* producer. Egypt imports seeds of some squash genotypes that need agricultural production. The main objectives for squash breeders are earliness, productivity, tolerance or resistance to biotic and abiotic stress, and fruit quality (Loy, 2004).

UV-B irradiation (280-315 nm) when applied on plants can cause damage in the DNA structure of the plants. It induced reduction in stem length and number of floral (Kumari et al., 2021). Squash cultivars varied among genotypes in the number of male flowers per plant, number of fruits developed per plant, average weight of fruit, fruit diameter, number of seeds per fruit, and the content of chlorophyll a. Exposure period of 30 min irradiated squash cultivars with ultraviolet rays (UV-C) significantly increased the number of male flowers and chlorophyll content. The few exposure time to UV rays may cause the plant to be resistant to oxidative stress generated by UV rays by increasing antioxidants as phenols and proline, leading the plant more resistant to environmental stress (Hammok and Esho, 2022). In addition, Singh et al. (2022) found early flowering and early edible fruit maturity in brinjal under 5 KR of gamma irradiation. Under these doses of gamma rays, the fruit yield per plant was increased but under the increasing concentration of mutagen; the plants showed an adverse effect for all growth and yield traits among genotypes. Agronomic traits in potatoes treated with low doses of UV observed yield increased slightly. Similarly, photosynthetic and physiological traits were significantly increased. In addition, the synthesis of tuber nutrient as flavonoids, anthocyanins, vitamin C, phenols and chlorogenic acids were increased due to the expression of their structural genes. Meanwhile, the higher doses of UV-B irradiation caused greater damage in the pigmentation traits making the plants reduce the yield and tuber nutrients (Wu et al., 2023). In Balsam apple, Bammanakatti et al. (2023) observed high heritability for the number of fruits per plant in M_1 and M_2 generations, whereas fruit yield per plant showed positive association with fruit length and days to flowering. Meanwhile, the number of fruits per plant, fruit weight, fruit length, and fruit girth showed highest positive effect on fruit yield per plant.

Therefore, this study attempts to partition the variance components for morphological, physiological, and yield components in M_1 generation of summer squash released from UV-irradiated genotypes. These estimates are very important in developing reliable selection indices.

MATERIALS AND METHODS

The experiment was conducted at the experimental Agri Farm of Genetic Department inside the campus of Mansoura University

Table 1. Summer squash genotypes used in this study.

Genotype	Designation
Alexandarani	Genotype 1
1116228	Genotype 2
1116232	Genotype 3
1116237	Genotype 4

Table 2. Expectation mean squares derived from the analysis of variance.

Source of variations	Degrees of freedom	Mean squares	Expectation mean squares
Replications	r-1	MSr	$\sigma^2e + r\,\sigma^2g + g\,\sigma^2r\,\sigma$
Genotypes	g-1	MSg	$\sigma^2e + r\,\sigma^2g$
Error	(r-1) (g-1)	MSe	σ^2e

r = Number of replications, g = Number of genotypes, MSe = Error mean square, MSg = Genotypic mean square, MSr = Replications mean square, σ^2e = Error variance, σ^2g = Genotypic variance, σ^2r = Replication variance.

during the successive season of 2022, to study the effect of UV irradiation on the genetic variability of vegetative growth, sex expression, pigment concentration in leaves and fruits, as well as yield and its components. All agricultural practices of *C. pepo* L. were carried out as recommended by Egyptian Ministry of Agriculture.

Genetic material

Four genotypes of summer squash were used in this study (Table 1). Seeds of these genotypes were kindly provided by the gene bank of Agricultural Research Center, Giza, Egypt.

Ultraviolet irradiation

Seeds of all genotypes were soaked for 12 h before UV-irradiation according to Ehrenberg (1961). Irradiation was done in the laminar cabinet supported with UV lamp as an artificial source of UV rays which is present in the Laboratory of Microbial Genetics, Faculty of Agriculture, Mansoura University. The spectrum of UV-radiation usually used in mutation is high energy named UV-B (280-320 nm) (Barta et al., 2004). The spectrum of UV lamp used in this study was 300 nm, therefore it was classified as UV-B. Each minute of exposure to UV-radiation generated 188.2 joules/m^2 according to Kondrateva et al. (2020). The joules are defined as the amount of energy extracted when a force of one newton is applied over a displacement of 1 m which is equivalent to 1 W of power radiated for 1 s.

Experimental design

The seeds of all genotypes were irradiated with the laminar UV lamp at room temperature of 20 ± 3 for 0, 4, 8 and 12 min. By the end of treatment, seeds were sown directly into the soil in a randomized complete block design with three replicates. Each plot consisted of 16 ridges with 80 cm width and 3 m length. The seeds were sown on one side of ridge with 50 cm apart. The plants in each experimental plots were fertilized with the chemical fertilization of urea nitrogen at a rate of 320 kg of nitrogen/ha, as well as all agricultural service were applied from hoeing, weeding, controlling diseases, and insect infestations as recommended by the Egyptian Ministry of Agriculture in productive fields (Hammok and Esho, 2022).

Experimental measurements

After 45 days from sowing date, six plants were randomly chosen from each plot to measure the number of leaves per plant, number of male and female flowers per plant all over the flowering and fruiting period, chlorophylls pigments in leaves and fruits. Sex ratio was recorded by dividing the average number of female by male flowers. Fruits were harvested at two days intervals. During the fruiting period, the average fruit weight was recorded. Fruit yield was calculated as the number of fruits and the weight of fruits per plant. Sex ratio was measured according to Shafeek et al. (2016). Flowering traits and fruits yield were measured according to Abou El-Salehein et al. (2019). Photosynthetic pigments content in leaves and fruits were measured according to Arnon (1949). Plant height was measured at the end of plant life when the plants became to blooming according to Hassan et al. (2016).

Statistical analysis

The data were subjected to statistical analysis of variance procedure (Table 2), according to Snedecor and Cochran (1980).

Estimation of genetic parameters

The genetic parameters included genotypic variance (σ^2g), phenotypic variance (σ^2P), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), as well as environmental coefficient of variation (ECV) estimated according to Burton (1952) and Singh and Chaudhury (1985). Meanwhile, heritability (H) in broad sense and expected genetic advance (EGA) were estimated according to Allard (1960). The genetic advance assumed selection intensity of superior plants up to 0.05 of population. Meanwhile, the genetic advance as the percentage of

Table 3. Assessment of genetic parameters related to the number of leaves per plant in summer squash.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	038.41	042.55	004.14	90.27	031.87	059.42	27.55	12.12	40.24
4 min	350.94	481.51	130.57	72.88	160.64	188.16	27.52	32.93	96.90
8 min	028.47	054.58	026.11	52.16	048.43	067.05	18.62	08.35	27.51
12 min	023.98	135.89	111.91	17.64	037.94	090.32	52.38	04.24	10.18
Mean	110.45	178.63	068.18	58.24	069.72	101.23	31.51	14.41	43.71

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

population mean (GAM) in which selection apples was assessment according to Johnson et al. (1955).

RESULTS AND DISCUSSION

Variability is the phenotypic differences among individuals within the population due to variations in their genetic composition. The magnitude of variation in populations was important for effective selection. The knowledge about nature and magnitude of diversity in plant breeding materials are very significant for the improvement of summer squash. The crop shows high degree of genetic variations due to the high percentage of out crossing. The genetic gain from selection depends upon the genetic variations, as well as on the magnitude of the heritable portion of these variabilities. Induced mutations as carried out in this study have been used to generate genetic variations in summer squash populations to be used in improving yield and its components in this crop. The variability observed in summer squash populations resulted from genetic and environmental causes, of which only the genetic component is heritable. Furthermore, assessment of heritability alone do not indicate information about the expected gain of genetic advance in the next generation, but needs in conjunction to measure the genetic advance, the change in mean value between generations.

Number of leaves

As shown in Table 3, estimated range of genotypic variance for the number of leaves per plant ranged between 23.98 and 350.94. The genotypic variance is relatively much higher than the environmental variance, indicating the greater share of genotypic variance in the total variability. Greater genotypic variance than environmental variance indicated that the genotypic effect was high for the expression of genes controlled the number leaves developed per plant. Among the number of leaves per plant, genotypic variance values exceed 20%, regarded as high effects of genotypes on the gene expression of this trait. These results indicated that this trait offered high scope of selection, as this under the influence of genotypes. These results agreed with Ahmed et al. (2008), who found high level of genotypic variance for days to maturity, spikelets per spike, grains per spike, grain-filling period, and harvest index in barley. Meanwhile, the mean of phenotypic variance (178.63) was greater than the genotypic variance (110.45) with relatively small difference which indicated that the environmental effect was lower than genotypic effect for the gene expressed the number of leaves developed per plant. Therefore, the mean of genotypic coefficient of variation was greater than the mean of environmental coefficient of variation. This indicates lower influence of environmental factors than genetic factors on the expression of leaves number developed per plant. This disagreed with Nechifor et al. (2011), who decided that high difference between genotypic coefficient of variation and phenotypic coefficient of variation is due to great influence of the environment on this trait. In this respect, Deshmukh et al. (1986) categorized phenotypic coefficient of variations as low (10%), medium ranged from 10 to 20%, and high exceeds 20%.

Heritability estimates gives insight about the extent of genetic control on the expression of trait under investigation, as well as phenotypic reliability in predicting the related breeding value (Nahar et al., 2016). Estimated heritability in broad sense ranged from 17.64 to 90.27. As decided by Robinson et al. (1949), heritability was characterized as low (0-30%), moderate ranged from 30 to 60% and high exceeds 60%. The mean of heritability obtained in this study for number of leaves per plant was equal 58.24% categorized as moderate heritability, based on the aforementioned classification. The genetic advance as percentage of means in combined analysis ranged from 10.18% at the dose of 12 min exposure to UV to 96.90% at the dose of 4 min exposure to UV. Johnson et al. (1955) reported that genetic advance as percentage of mean can be categorized as low ranging from 0 to 10%, moderate ranging from 10 to 20%, and high above 20%. The mean of genetic advance as percentage of means in combined analysis for the

Table 4. Assessment of genetic parameters for leaf area per plant in summer squash.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	0.21	1.64	1.43	12.80	15.31	42.78	27.47	0.34	43.83
4 min	0.01	0.25	0.24	4.43	03.48	16.51	13.03	0.05	01.98
8 min	0.51	1.22	0.71	41.80	05.99	14.34	08.35	0.94	44.47
12 min	0.61	0.92	0.31	66.30	24.41	29.98	05.57	1.31	51.42
Mean	0.34	1.01	0.89	31.33	12.29	25.90	13.61	0.66	35.43

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

number of leaves per plant equal 43.71%, classified as high value depending on the classification of Johnson et al. (1955). These results indicated that moderate value of heritability was coupled with the high value of genetic advance as a percentage of means. Therefore, selection depending on the phenotypic performance of genotypes leads to increase in the mean performance of the selected individual plants for the number of leaves per plant. The results obtained herein agreed with Nechifor et al. (2011), who demonstrated moderate values of heritability and genetic advance for the number of seeds per pod in common bean. In addition, the low values of heritability reflected the influence of environmental factors on the trait under investigation that limit the scope of improvement through selection.

Leaf area

The results presented in Table 4 revealed that the genotypic coefficient of variation ranged between 0.01 and 0.61. Meanwhile, the phenotypic coefficient of variation ranged from 0.25 to 1.64. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation. The larger magnitude of difference between the two parameters indicates higher influence of environmental factors than the genetic elements in the expression of leaf area per plant. Therefore, selection depending on the phenotypic performance may not be appropriate for the leaf area. This indicated that the environmental factors played a considerable role on the gene expression of leaf area. Heritability percentage ranging from 4.43 to 66.30% produced the mean of heritability equals 31.33%, categorized in general as moderate heritability. Meanwhile, the phenotypic coefficient of variations is greater than the genotypic coefficient of variations for the leaf area per plant. Though, the environmental effects are predominance on the expression of genes controlled the leaf area per plant. In addition, the results showed that high difference between genotypic and phenotypic coefficient of variations reflected higher influence of environmental effects on the gene expression of leaf area. The mean of phenotypic coefficient of variations concerning leaf area is categorized as high (25.90%) because its value exceeds 20%. The estimated genetic advance as percentage of mean ranged from 1.98 to 51.42% which produced the mean value equals 35.43%.

Based on the aforementioned classification, the estimated genetic advance for leaf area per plant was high because its value exceeds 20%. Moderate heritability obtained in leaf area was coupled with high genetic advance suggesting that improvement would be partially effective through phenotypic selection. According to Blanco and Folegatti (2005), leaf area is a key variable for plant growth, photosynthetic efficiency, yield indicator, and irrigation responses. The results obtained herein are in line with Raju et al. (2002), who stated that high heritability coupled with high genetic advance in percentage of mean found in parents and hybrids of summer squash suggested that improvement would be effective through phenotypic selection.

Genetic components of female and male flowers

The results presented in Table 5 showed that the genotypic coefficient of variation for the number of female flowers per plant was higher than the environmental coefficient of variations.

Therefore, the differences between genotypic coefficient of variations and phenotypic coefficient of variations were relatively large in magnitude of female flowers developed per plant, indicating high influence of environmental effects in the expression of this trait. These results agreed with Fekry (2016), who found that ethephon recorded the best results in floral characteristics via decreasing the number of male flowers, as well as increased the number of female flowers, femaleness and yield components in summer squash. Moreover, Shafeek et al. (2016) stated that foliar application of etheral on summer squash with high level concentration (150 mg/L) induced greater number of female flowers developed per plant when compared with the control. In addition, Costa-Silva et al. (2020) found that ethylene can change the flowering pattern in zucchini

Table 5. Assessment of genetic parameters for female flowers developed per plant in summer squash.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	05.52	21.24	15.72	25.99	32.45	63.67	31.22	2.47	18.85
4 min	05.31	14.32	9.01	37.08	30.55	50.17	19.62	2.89	20.31
8 min	16.60	19.79	3.19	83.88	53.80	58.74	04.94	7.67	53.51
12 min	08.16	29.19	21.03	27.95	45.19	85.47	40.28	3.11	31.12
Mean	08.89	21.14	12.24	43.73	40.49	64.51	24.02	4.04	30.95

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

Table 6. Assessment of genetic parameters for male flowers developed per plant in summer squash.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	11.23	23.19	11.96	048.43	15.30	56.21	40.91	04.80	26.20
4 min	13.08	67.33	54.25	019.43	43.24	98.10	54.86	03.28	18.76
8 min	12.03	29.69	17.66	040.51	43.07	67.67	24.60	04.55	28.06
12 min	13.62	17.63	04.02	129.44	35.98	40.94	04.96	11.17	42.49
Mean	12.49	34.46	21.94	059.45	34.39	65.73	31.34	05.95	28.88

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

squash via influencing the sexual expression but not enough to show complete sex reversal.

Moderate heritability (43.73%) was obtained for the number of female flowers developed per plant coupled with high genetic advance in percent of mean (30.95%), suggesting that environmental factors play a considerable role in expressing female flowers developed per plant. These results indicated that moderate heritability of female flowers developed per plant is due to the influence of environmental factors that limits the scope of improvement by selection based on phenotypic performance of genotypes. Increasing female flowers leading to increasing squash yield which is the main target for growers. In this respect, Baruah and Sarma (2018) found that application of ethrel on *Cucumis sativus* L. improved flowering behavior via increasing the number of female flowers.

Estimated genetic components of male flowers developed per plant shown in Table 6 showed that the differences between genotypic and phenotypic coefficient of variations exhibited large values in magnitude of male flowers developed per plant. The estimated environmental coefficient of variation ranged between 4.96 and 54.86% indicating the higher effects of environmental factors than genetic factors in the gene expression developed male flowers. The heritability of this trait has a moderate value (59.45%) depending on the classification of Robinson et al. (1949). The mean of genetic advance as a percentage of means in combined analysis was high (28.88%) depending on the classification of Johnson et al. (1955). The results indicated moderate values of heritability coupled with relative increase in genetic advance for the number of male flowers developed per plant. Therefore, the results stated that moderate heritability estimates reflected high effect of environmental factors than genetic factors on the phenotypic expression of male flowers developed per plant, as well as the effectiveness of selection in improving this trait. Heritability demonstrated whether differences observed on the level of individuals arose as inducement by differences in the genotypes or due to the environmental factors.

Thus, Singh (2001) stated that a close correspondence between the genotypic and phenotypic performance is due to small contribution of environmental factors on the phenotype. In addition, traits with lower heritability leading to selection may be considerably difficult due to the masking effect of the environmental factors.

Chlorophylls in leaves

As shown from the results presented in Table 7, the differences between the mean of genotypic coefficient of variation (12.04%) and phenotypic coefficient of variation (22.12%) were relatively small, indicating that the

Table 7. Estimates of genetic parameters for chlorophyll concentrations in leaves of summer squash genotypes.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	0.05	0.196	0.150	25.51	11.83	23.43	11.60	0.23	025.98
4 min	0.03	0.110	0.080	29.09	10.39	19.28	8.89	0.19	026.72
8 min	0.15	0.155	0.008	94.94	06.02	25.19	19.17	0.76	123.02
12 min	0.07	0.073	0.005	96.84	19.94	20.59	0.65	0.54	124.79
Mean	0.07	0.133	0.060	61.59	12.04	22.12	10.08	0.43	075.13

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

Table 8. Estimates of genetic parameters for fruit chlorophylls concentration in summer squash genotypes.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	0.0012	0.0018	0.0006	81.21	04.94	06.08	1.14	0.071	057.54
4 min	0.0013	0.0033	0.0022	39.39	05.55	06.79	1.24	0.047	029.41
8 min	0.0042	0.0055	0.0013	76.36	10.58	12.11	1.53	0.116	123.83
12 min	0.0020	0.0030	0.0010	66.66	06.45	07.90	1.45	0.074	061.80
Mean	0.0002	0.0007	0.0013	65.90	06.88	08.22	1.34	0.077	068.14

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

observed differences in this trait were mostly due to the genotypes, but the environmental factors played a limited role in the gene expression of chlorophyll formation. Similar results were noticed earlier by Abebe et al. (2017), who observed the narrow magnitude of variation between phenotypic and genotypic coefficient of variance in rice cultivar for days to maturity, culm length, plant height, panicle length and biomass production, reflected limited influence of environmental factors in the expression of these traits. Thus, selection based upon phenotypic performance of chlorophylls concentration in leaves would be effective to bring about considerable genetic improvement.

Therefore, the mean of heritability estimates of chlorophyll concentration in leaves was relatively greater than 60% (61.59%) categorized as high heritability percentage depending on the classification of Robinson et al. (1949). High values of heritability estimates were coupled with high genetic advance (75.13%) as a percentage of mean. This indicated that selection depending on phenotypic performance of the genotypes leads to increase in the mean performance of selected progenies for photosynthesis.

Chlorophylls and carotenoids in fruits

As shown from the results tabulated in Table 8, the differences between phenotypic and genotypic coefficient of variations concerning chlorophylls concentrations in fruits were relatively small, indicating that the observed variations of this trait were mostly due to genetic elements but environmental factors were also showed a decreased role in the expression of this trait. Therefore, the heritability of this trait categorized a high value (65.90%) based on the classification of Robinson et al. (1949), who categorized heritability as high when its value reached 60% and above. High heritability obtained in this study for chlorophylls concentrations in fruits coupled with high genetic advance (68.14%) as a percent of mean.

Therefore, selection based on phenotypic expression of genotypes leads to the exhibition of the mean expression of selected progenies. The results obtained herein are in line with Manju and Sreelathakumary (2002), who decided the effectiveness of selection based on genetic advance of the selected trait along with heritability. In addition, Hailu et al. (2016) found high heritability coupled with moderate high genetic advance based on the percentage of mean for the number of productive tillers/m^2 in barley length of spike, number of kernels/spike, as well as harvest index among locations. The results obtained in this study indicated the involvement of additive gene action in the inheritance of chlorophylls concentrations in fruits.

The results tabulated in Table 9 showed that the

Table 9. Estimates of genetic parameters for carotenoids concentration in fruits of summer squash genotypes.

Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	0.006	0.370	0.360	01.63	04.39	11.76	07.37	0.020	02.66
4 min	0.040	0.120	0.080	30.00	09.57	17.47	07.90	0.210	21.44
8 min	0.008	0.039	0.031	45.29	06.23	13.76	07.53	0.180	35.35
12 min	0.030	0.136	0.110	19.11	10.19	23.32	13.13	0.145	23.13
Mean	0.021	0.166	0.145	23.94	07.59	16.58	08.98	0.138	20.64

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

Table 10. Estimates of genetic parameters for fruits weight per plant in summer squash genotypes

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Doses of UV irradiation (kGy)	GV	PV	EV	H (%)	GCV (%)	PCV (%)	ECV (%)	EGA	GAM (%)
Control	0.083	0.163	0.080	50.92	12.00	16.00	4.00	0.419	29.64
4 min	0.090	0.180	0.090	50.00	12.13	17.14	5.01	0.459	30.02
8 min	0.210	0.260	0.050	80.76	18.21	20.27	2.06	0.850	53.71
12 min	0.190	0.350	0.160	54.28	21.29	28.90	7.61	0.850	90.52
Mean	0.140	0.240	0.095	58.99	15.91	20.58	4.67	0.640	50.97

GV, Genotypic variance; PV, phenotypic variance; EV, environmental variance; H (%), heritability in broad sense in percent; GCV (%), genotypic coefficient of variation in percent; PCV (%), phenotypic coefficient of variation in percent; ECV (%), environment coefficient of variation in percent; EGA, expected genetic advance; GAM, genetic advance as percent of mean at 5% selection intensity; kGy, kilo Gray of rays.

differences between genotypic and phenotypic coefficient of variations were relatively high in magnitude of carotenoid concentrations in fruits, indicating higher effects of environmental factors than genetic background in the expression of this trait. The considerable difference obtained between phenotypic and genotypic coefficient of variation for carotenoids concentration in fruits indicates a greater effect of environmental conditions on the phenotypic expression of these traits. Therefore, selection based on the phenotypic expression of this trait would be ineffective to bring genetic improvement in the genotypes considered in this study. In addition, heritability values (23.94%) categorized as low (0-30%) based on the classification of Robinson et al. (1949).

In contrast, the mean of genetic advance as a percent of means reached 20.64% which is categorized as high (20% and above) depending on the classification of Johnson et al. (1955). Therefore, the low heritability obtained for carotenoids concentrations in fruits is due to the influence of environmental factors that limits the scope of improvement via selection. These results are in harmony with Dursun (2007), who obtained low board-sense heritability estimates for grain yield in *Phaseolus vulgaris* L. In addition, Alemayehu (2010) found moderate estimates of heritability and genetic advance for the number of seeds per pod in *Phaseolus vulgaris* L. Therefore, selection depending on the phenotypic expression of carotenoid concentrations in fruits limits the scope of improvement in the progenies because this trait is mainly influenced by the environment than genetic factors.

Fruits weight

The results presented in Table 10 showed that the differences between genotypic and phenotypic coefficient of variations were relatively moderate in magnitude of fruits weight per plant.

This indicated high influence of the environmental factors than genotypes in the expression of fruits weight per plant. The estimates of heritability (58.99%) categorized as moderate (30-60%) depending on the classification of Robinson et al. (1949). Moderate estimates of heritability obtained in magnitude of fruits weight per plant coupled with high genetic advance (50.97%) as a percentage of mean in combined analysis. Therefore, selection depending on the phenotypes leading to increase in fruits weight per plant in the offspring of selected progenies. These results agreed with Ejara et al. (2018), who found that plant height, as well as, seeds number per pod in common bean (*Phaseolus vulgaris* L.) showed moderate heritability coupled with relatively high genetic advance based on the percentage of mean.

As opposite to the results obtained herein, Hailu et al.

(2016) found that days to maturity in barley, as well as days to heading depicted high heritability values with lower genetic advance. These traits possessing high heritability with low genetic advance indicating the influence of non-additive gene action. Therefore, selection technique in early segregating generations will not be effective for improving these traits. Further studies by Sardana et al. (2007) found that high values of heritability might not be necessary to increase genetic advance gain through selection, unless sufficient genetic variations existed in the genome. In addition, Chand et al. (2008) stated that high values of heritability with low genetic gain were obtained for days needed to ear emergence in barley.

In conclusion, heritability reflected whether differences shown within individuals arose as a result of variations in the genotypes or due to the environmental factors. High heritability values coupled with high genetic advance indicated that selection is effective based on the phenotypic performance. High heritability coupled together with relatively greater genetic advance as a percentage of the mean indicated the involvement of additive gene action in the inheritance of this trait leading to selection will be effective. However, low genetic advance with high heritability estimates indicated the involvement of non-additive gene action leading to selection will not be effective. The reason for low heritability arose from some variance constituting the environmental variance indicating slow progress based on selection for this trait.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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