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Genetic relationship in Vigna radiata(L.) based on morphological traits and Biochemical traits

DISSERTATION-II

SYNOPSIS

Submitted by:

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In partial fulfillment for the award of the degree
Of
Masters of Science in agriculture
(Genetics and Plant Breeding)

Under Guidance of
Dr. Sanjeev Kumar
(Assistant Professor)

Discipline of Agriculture
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CERTIFICATE

This is to certify that the project entitled study of "**Genetic relationship in *Vigna radiata (L.)* based on morphological traits and Biochemical traits** " is going to be done by Shelly Sansam (11717522), as per the research work (Dissertation program, GPB 596) in partial fulfillment for the award of the degree of Masters Of Science in Agriculture (Genetics & Plant Breeding) from Lovely Professional University, Phagwara, Punjab under the guidance of supervisor.

Dr. Sanjeev Kumar

(Assistant Professor)

Lovely Professional University, Phagwara

DECLARATION

Certified that this report is an overview of practical work which I am going to perform. It is my individual research under the project entitled "**Genetic relationship in *Vigna radiata(L.)* based on morphological traits and biochemical traits** " going to complete during the academic period 2017-2019 of M.sc. Agriculture (Genetics & Plant Breeding) course, supervising and guiding by Dr. Sanjeev Kumar, Assistant Professor at Lovely Professional University, Phagwara, India.

I also declare that this project will be done in this university only and it shall not be submit to any other university for the award of any degree.

Shelly Sanasam(11717522)

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INTRODUCTION

The total world population is projected growing from current 7.49 billion (in March 2017) to 8.9 billion by 2050 (United Nations Report 2004).Therefore, to provide feed for increasing world population required ample quantity of food. It is also noted due to nutritional security increasing food production is a major challenge. To fulfil the demand of quality and overall nutritional security becomes more important and overcome these challenges by promoting food/grain legume crops, diversifying the global cropping pattern and generally called as Pulses. Green gram [*Vigna radiata*(L) Wilczek] is an annual, erect or semi-erect plant with a height of 0.15-1.25 m (Lambrides et.al. 2006; Mogotsi 2006). It is slightly hairy and possesses well-developed root system. Wild types tend to be prostrate while cultivated types are more erect (Lambrides et al.,2006 Green gram[*Vignaradiata*(L) Wilczek] is an annual, erect or semi-erect plant Generally crop is grown as rain fed but under assured irrigation in Indo Gangetic plains of Northern India grown in summer.

The productivity status for major pulse crops may very low in contrast with that of cereals, which have been selected for high grain yield under high input conditions. Among various factors hampering realization of actual yield potential of pulses, is lack of genetic diversity, poor harvesting index and susceptibility to disease and pest. In spite of all the efforts, development of sustainable cultivar with higher yields has not yet been successful due to narrower genetic bases of the present cultivar. An assessment of genetic diversity in available cultivars is an essential pre-requisite for understanding the progress, made in any breeding programme. Recently, in Punjab region, less production from pulses is due to lack of germplasm availability that will responsible for high yielding production from pluses. In India, faces real problems with pulses are the low productivity of existing strains, an absence of technological progress and the inability of modern science to address the issues of high vulnerability of pulses to pests and diseases. Possibilities of high yielding germplasm is minimal in case of pulses whereas in case of cereals such as wheat and rice, could be obtained from overseas for breeding in India-specific crop varieties. Yield is being a quantitative complex character and it could be improve with the cross between the parents with the maximum genetic divergence, which is generally responsive for genetic improvement (Arunachalam, 1981).Green gram has been known for its high protein content, it is a main source for vegetarian's protein as it helps to reach the daily requirement.

Albumin and Globulin are the main proteins present in Green gram . Very few studies have been carried out on the nutritional quality of green gram proteins. Supplementation with methionine has been shown to improve the nutritive value of raw and autoclaved green gram 1.3 to 3.1 and 1.3 to 3.4 respectively (Khader, 1996). Mungbean protein isolate was acylated to various degrees by acetic and succinic anhydrides. Changes in functional properties (protein solubility index in different solutions, water and oil absorption capacities, emulsification properties, foam capacity and stability), antinutritional factors (tannins, phytic acid and trypsin inhibitor) and in-vitro protein digestibility of acylated protein isolate were determined (El-Adawy, 2000). The effects of some domestic traditional processes, such as dehulling, soaking, germination, boiling, autoclaving and microwave cooking, on the nutritional composition and antinutritional factors of mung bean seeds were studied. Germination and cooking processes caused significant ($p<0.05$) decreases in fat, carbohydrate fractions, antinutritional factors and total ash contents. All processes decreased the concentrations of lysine, tryptophan, threonine and sulfur-containing amino acids. However, all treatments were higher in total aromatic amino acids, leucine, isoleucine and valine contents than the FAO/WHO reference. Dehulling, soaking and germination processes were less effective than cooking processes in reducing trypsin inhibitor, tannins and hemagglutinin activity contents. Also, germination was more effective in reducing phytic acid, stachyose and raffinose. Germination resulted in a greater retention of all minerals compared to other processes. In vitro protein digestibility and protein efficiency ratio were improved by all processes. The chemical score and limiting amino acids of mung bean subjected to the various processes varied considerably, depending on the type of process. (Mubarak,2005). Isolated protein from green gram and the chemical composition was determined. It contained 64.04% protein, 1.8% total lipids, 27.64% total carbohydrates, 1.68% crude fibre and 4.84% ash. Iron, calcium, magnesium, copper, zinc, potassium and sodium were determined. The limiting amino acid in the protein isolate was lysine. In vitro digestibility pepsin followed the pancretion was the highest and the lowest was the digestion by pepsin alone. Water absorption, oil absorption, emulsion capacity and nitrogen solubility index (NSI) of the protein isolate were 2.26g/g, 1.24g/g, 31.4g/g and 6.8g/g, respectively (Mesallam and Hamza, 1986).

Our present study is mainly focused to explore the germplasm in Punjab condition by identifying potential trait from diverse germplasm collected from different agro climatic zones of India. Potential germplasm may response well in the climatic condition and provide good results for yield related attributes. Genetic diversity is used to check the genetic bases of the cultivars that can be utilized as parental lines for further breeding programmes.

OBJECTIVES

This study main aim is to screen different cultivars of green gram and see the diversity among all the collected germplasm and estimate their biochemical property (seed protein) for understanding the compositional difference and variability among all the different genotypes and select the best cultivar which will be economical and have high nutrient content.

1. To estimate genetic diversity of selected *Vigna radiata* using phenological characters.

2. Analyze genetic diversity among *Vigna radiata* using seed protein profiling.

3. Congruent study morphological and biochemical characters of selected cultivar of *Vigna radiata.*

REVIEW AND LITERATURE

Pulses production in the country has witnessed a significant aspect during the last decade due to development of high yielding disease resistant varieties using recombinant breeding programmes and their after adoption of such improved pulse production technologies by farmers. However, the realized yield of pulses is far from their actual potential. Among various factors hampering realization of actual yield potential of pulses, "Genetic diversity" is an important one. In view of the substantial yield losses caused by genetic diversity in pulses it is imperative to develop recombinant with diverse genetic basis by understanding the usage of statistical tools at field level and laboratory tools requirement to identify the diverse germplasm in order to increase in genetic variability with desirable background.

Datta and Sony, (2012); Krishnan(2014), evaluated different genotypes of Green gram using morphological and molecular basis for yield and yield related components such as plant height, Days to maturity, Pod length , number of pod /Plant, Number of clusters and disease resistant ,resistant to insect pest etc. Results obtained from these datasets that they screened potential cultivar of green gram under local condition and that can be evaluated for future crop improvement used in breeding programme.

Pandey (1981), reported that the number of pods per square meter was most affected by water stress in all four species, followed by number of seeds per pod, while seed weight was least affected. Harvest index decreased linearly with increasing levels of drought for all four species.

.**Katiyar et.al, (2008),** studied during 2004-06 on morphological characterization of greengram [Vigna radiata (L.) Wilezek] and establish distintness of the candidate variety from all other varieties. A total of 73 released Indian cultivars of greengram were grouped for several agromorphological descriptors. The anthocyanin colour was present in all varieties except in 'Pant M 3', 'OBGG 52', 'RMG 344', 'COGG 912' and 'HUM 12'. The stem colour was generally green or greenish with purple splashes except in 'AKM 8802' which depicted purple colour. Wide diversity (38 to 70 cm) has been observed in plant height. Shiny seed lusture was observed in 'IPM 99-125 (Meha)', 'GM 3', 'Asha', 'MUM 2', 'Samrat (PDM 139)', 'PDM 11', 'Pusa 105', 'PS 16', 'RMG 268', 'Sujata' and 'Sona'. All the greengram varieties are green in seed colour except 'Sona' which is yellow in colour. Maximum varieties are of medium seed size (3-5 g/100-seed weight) except 'Pusa Vishal'

and 'SML 668' (large seeded > 5 g /100 seed). Such characterization of released cultivars will provide valuable information for the strengthening of further breeding programme in greengram.

Ramakrishnan (2014), investigated the principle component and cluster analysis and Pearson's correlation analysis for yield and yield related traits to tailor the genetic diversity of mungbean

Hanna et al.,(1999), studied the efficiency of a breeding programme for the improvement of quantitative traits depends to a large extent on magnitude of variability present in the available germplasm

Bisht et. al. (2008), analysed genetic diversity and patterns of variation in Green gram among 111 accessions representing the better agronomic types from the entire collection of the National Bureau of Plant Genetic Resources were studied. Wide variation in morphological and agronomic traits *viz.* number of pods per plant, yield per plant, 100-seed weight, fruit setting capacity, flowering period, maturity, number of pod bearing peduncles, plant height, number of primary branches, length of branch, nodulation and leafiness was observed. Grain yield was found to be significantly correlated with 100-seed weight and pod length. The data were subjected to cluster and principal components analysis. The accessions were grouped into six discrete and well-defined clusters. The study demonstrated the patterns of variation at the population level. The multivariate analysis was useful in identifying a group of accessions with yield enhancing traits within a highly diverse group of accessions and their potential value in green gram improvement is suggested.

Mesallam and Hamza, (1986), Isolated protein from green gram and the chemical composition was determined. It contained 64.04% protein, 1.8% total lipids, 27.64% total carbohydrates, 1.68% crude fibre and 4.84% ash. Iron, calcium, magnesium, copper, zinc, potassium and sodium were determined. The limiting amino acid in the protein isolate was lysine. In vitro digestibility pepsin followed the pancretion was the highest and the lowest was the digestion by pepsin alone. Water absorption, oil absorption, emulsion capacity and nitrogen solubility index (NSI) of the protein isolate were 2.26g/g, 1.24g/g, 31.4g/g and 6.8g/g, respectively.

Johns and Waterman (1920), reported the mung bean contains 21.74% of protein. A series of experiment made with aqueous solution of sodium chloride indicated 5% as the most effective concentration of this salt for the extraction. From the finely ground seed twenty volumes of 5% sodium chloride solution dissolved about 19% of protein, calculated on the dry weight of the meal used or, 87.5% of the total protein. A very low coagulation, together with two others at higher temperatures, indicated an albumin and two globulin fractions.

Coffmann and Garcia (1977), evaluated a protein isolate was prepared from mung bean flour by extraction with0.001 N NaOH, precipitation at pH 4.5, neutralization of the dispersed precipitate to pH 6.8-7.0, and subsequent freeze drying. The isolate's amino

acidcomposition was determined and found to be similar to that of mung beanflour except for cystine which was destroyed during isolate preparation. The following properties of the protein isolate were investigated: nitrogen solubility,buffer capacity, foamability, gelation. Except for buffer capacity, the isolate demonstrated good functional abilities in simple systems under laboratory conditions.

El-Adawy (1996), reported Oat contains cereal protein globulin and also legume protein avenalin, as the major protein (80%), It is twice richer in protein, four times richer in calcium as compared to other grains (Gopalan et al., 1996). Mung bean is an excellent source of protein (2 7 %), a n d its essential amino acid composition compares favourably with that of soybean, kidney bean and FAO/WHO reference protein. The food legumes are major sources of protein and other nutrients in the diets of many developing countries.

A) STUDY AREA:

The present investigation was carried out under field condition in the "Agricultural Research Field, School of Agriculture, Lovely Professional University, Phagwara, Punjab - 144411.

1) Geographical location:

The Lovely Professional University is situated in the south of NH-1 (G.T. Road) in the district Jalandhar in the state of Punjab at a distance of about 350 km from Delhi. It is situated between 31o15' North latitude, 75o 42' East latitude at an altitude of 228 meters above the mean sea level (MSL). The district lies in the center of Punjab and is situated between two rivers Sutlej and beas. the soil of the village is alluvial and varies from coarse loamy to fine loamy. the total agriculture land of the district is 134 hectare. Major source of irrigation in tube wells, bore wells and then pump sets. Only 3% of land is irrigated by canal.

2) Climatic conditions:

The temperature reaches above 40 degree Celsius during summer and during winter the temperature goes down below 10 degree Celsius. The area falls under sub humid region. June being the hottest month and January being the coldest month. The average rainfall is 719mm. the highest rainfall is recorded during the month of July and the driest month is November.

B) MATERIALS AND METHODS

The present investigation entitle as "as **"Genetic relationship in *Vigna radiata* based on morphological traits and Biochemical traits"** will be carried out at field plot of Lovely Professional University, Phagwara, Punjab. Geographically, it falls under humid subtropical zone. The experiment will conducted at the field as well as Green house and laboratories of School of Agriculture, Lovely Professional University for morphological data recording as well as for genetical data recording. The details of material, Sources of materials, Experimental design, Statistical techniques and Laboratory tools and procedure that will use during the course of this investigation is presented as follows.

Experimental materials

The experimental material for present study consisted of 20 different characters of Green gram (*Vigna radiata*). The strains that selected for investigation will exhibit variation for important qualitative and quantitative characters to study diversity among them along with their morphological and biochemical traits.

Table 1: List of germplasm is given as follows:

S.NO	Name of Variety/Inbred line	Source	Characteristics
1	HUM-1	BHU, Varanasi	Spring and kharif season , Days to Maturity 60-65, Yield 9.4-16.0 q/ha
2	Gold	IIPR	Unknown
3	Moongi	IIPR	Unknown
4	HUM-1L	BHU, Varanasi	Summer season, Days to Maturity 60-62, Yield11.2 q/ha
5	PUSA-460	IARI, New Delhi	Unknown
6	MLA 720	PAU, Ludhiana	Unknown
7	Kopergane	BHU, Varanasi	Solid and Green bold seeds, Days to maturity 60-65, Yield 8-10q/ha
8	LM-5	BHU, Varanasi	Unknown
9	PUSA-Vishal	IARI, New Delhi	Summer season , bold seed , Days to Maturity 62, Yield 11.0 q/ha
10	LG-420	BHU, Varanasi	Unknown
11	IPM-2	IARI, New Delhi	Large seed suitable for rainy season, Days to Maturity 62-68, Yield 11-12 q/ha
12	SML-668	PAU,	Spring-Summer season , Tolerance to

		Ludhiana	MYMV, Days to Maturity 60-63,Yield 11.3 q/ha
13	JAUM0936	KVK,Manipur	Not yet known
14	KM2241	KVK,Manipur	Not yet known
15	ML2479	KVK,Manipur	Not yet known
16	PUSA0672	KVK,Manipur	Not yet known
17	NVL855	KVK,Manipur	Not yet known
18	SVM6133	KVK,Manipur	Not yet known
19	IPM512-1	KVK,Manipur	Not yet known
20	MH1323	KVK,Manipur	Not yet known
21	PUSaM 1772	KVK,Manipur	Not yet known
22	IPM410-9	KVK,Manipur	Not yet known

Observation record

Observation on plant will be recorded for germination, growth habit, days to flowering whereas the characters like plant height, number of branches, number of clusters, numbers of pod per cluster, total number of pods per plant, number of leaves will be taken for 12-15 plants randomly from each rows of each replication unit arranged with different genotypes.In the present investigation RBD will followed for field plot experiment and the replication wise mean values of the treatments will subjected to the following statistical analysis such as Analysis of variance (ANNOVA), Metroglyph analysis, Jacquard coefficient, Correlation coefficient

MARPHOLOGICAL DATA TO BE CONSIDERED

i) Days to 75% flowering:

Number of days required from date of sowing to the date on which 75% of the plants flowered was recorded.

ii) Days to reproductive phase:

Number of days required from days to 75%flowering to the date of maturity of plants was recorded.

iii) Days to maturity:

Number days required for the physiological maturity from the date of sowing were required.

iv) Plant height (cm):

At the physiological maturity the height of individual sampled plant were measured in centimetres from the ground level to the flower of the main shoot.

v) Number of primary branches per plant:

The branches of the observational plant like primary branches were counted and recorded at the time of harvest.

vi) Number of secondary branches per plant:

The branches of the observational plant like secondary branches were counted and recorded at the time of harvest.

vii) Number of clusters per plant:

The number of clusters per plant were counted and recorded after maturity.

viii) Number of pods per plant:

Numbers of pods per plant were to be counted at the time of harvesting

ix) Length of the pod (cm):

The length of the pod was calculated after harvesting all pods from the randomly selected plants.

x) Number of seeds per pod:

The seeds of different randomly selected plants were counted and average was taken out considering number of seeds per pod.

xi) Seed weight (g):

One hundred threshed grains will be take randomly after sun drying at 12% moisture level and weighted in gram with the help of electric balance.

xii) Yield per plant (g):

The seeds from different randomly selected pods of the chosen plants were taken out and counted and worked out average for number of seeds pod^{-1}.

xiii) Yield per plot (g):

The total seed was harvested from each plant of each genotype within each and every replication and weighted on an average.

SEED PROTEIN PROFILING :

Seed protein samples will be extracted from all genotypes by using standard protocol and quantify the samples by Lowry's Method. Protein profiling of the cultivars will be done utilizing SDS-PAGE method for the comparison of genetic diversity of genotypes based on the result.

STATISTICAL ANALYSIS:

The mean values of five randomly selected observational plants for ten different characters were used for statistical analysis. The following statistical parameters were calculated for presentation of data on different quantitative attributes.

Analysis of variance (ANOVA):

The analysis of variance (ANOVA) was calculated as proposed by Sukhatme and Panse (1985) in the following format:

The replication-wise mean values of different genotypes will subject to Randomized Block Design analysis of variance. The following model will use:

$Xij = \mu + ri + tj + eij$

Where,

Xij = Performance of jth treatment in the ith replication.

μ = General mean of the population.

ri = True effect of ith replication

tj = Effect of jth treatment.

eij = Random error associated with jth treatment in ith replication.

The data for different characters will analyse to test significance of difference among genotypes.

Estimation of variability:

By using Coefficient of Variance

$$C.V. = \sqrt{\text{error mean square}} \div x \times 100$$

Where, X = mean of character

The coefficient of variation (C.V.), being a unit less measurement, is a good basis of comparing the extent of variation between different characters with different scales among genotypes.

Randomized block design:

In the present investigation RBD will followed for field plot experiment and the replication wise mean values of the treatments will subjected to the following statistical analysis such as Analysis of variance (ANNOVA), Metroglyph analysis, Jacquard coefficient, Correlation coefficient

EXPECTED OUTCOME

The present study which will conducted by using 22 cultivars of green gram to study the genetic diversity based on its phenological and biochemical traits. The salient expected outcomes of the investigation are presented under the following sub-head:

1. Analysis of variance and mean values
2. Estimation of variability among selected germplasm of Green gram (*Vigna radiata*)
3. Assessment of correlation among characters/traits using correlation analysis
4. Estimation and identification of diverse Green gram(*Vigna* radiata) germplasm
5. Estimation of biochemical characters for checking their varietal difference among the germplasm grown in our field.

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