

**STUDIES ON MORPHOLOGICAL AND
BIOCHEMICAL CHARACTERS ATTRIBUTING SEED
YIELD AND QUALITY IN ONION (*Allium cepa* L.)**



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**Thesis submitted to the
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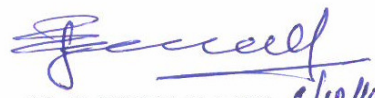
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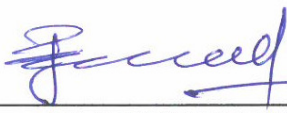
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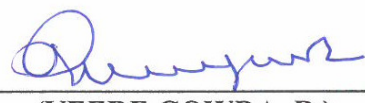
This is to certify that the thesis entitled “**Studies on morphological and biochemical characters attributing seed yield and quality in Onion (*Allium cepa* L.)**” submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE in SEED SCIENCE AND TECHNOLOGY** to the University of Agricultural Sciences, Bengaluru is a record of *bona fide* research work done by **Mr. JYOTHI NARAYANA, A. PALB 4267** during the period of his study in this university under my guidance and supervision. The data of this thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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A c k n o w l e d g e m e n t

This is the most difficult job to accomplish for, poor a verbalist I am to express all my feelings and sense of gratitude in words. I cannot just satisfy myself with but, for the limitation of space, I have got to put my vote of thanks to everyone who has directly or indirectly shared views with or contributed in materializing my investigation and degree program.

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My hat is off to the following members of advisory committee who have justified their honourable job with eminent piece of advice.

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Bengaluru
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(Jyothi Narayana, A)

**STUDIES ON MORPHOLOGICAL AND BIOCHEMICAL
CHARACTERS ATTRIBUTING SEED YIELD AND QUALITY IN
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JYOTHI NARAYANA, A.

ABSTRACT

Onion is one of the most important commercial vegetable crops, having high potential for seed production. In the recent years onion production is mainly carried out through direct seeding. To achieve better seed quality, identifying characters that are associated with seed quality is need of an hour. Hence, an investigation was conducted to study the morphological and biochemical characters attributing seed yield and quality in onion. In the study 14 genotypes of onion were evaluated for 36 different characters. The study revealed that, wide range of variability and high heritability for all the characters studied. Phenotypic coefficient of variation was found to be more than genotypic coefficient of variance in respect to all the characters studied. Correlation studies revealed that characters *viz.*, number of leaves, plant height, days to flower initiation, bulb weight, total soluble sugars, total soluble proteins, α -amylase activity showed more positive association with seed quality. While Characters *viz.*, seed yield per plant, bulk density and number of seeds per umbel posed negative direct effect on most of the seed quality attributes.

Characters having high positive correlation also showed high heritability and genetic advance, confirming their practical applicability in breeding methods. Two hybrids Arka Lalima and Arka Kirthiman showed high mid parent, better parent and standard parent heterosis for characters like total soluble sugars, total soluble proteins, α -amylase activity and germination after controlled deterioration.

December, 2016
Department of Seed Science and Technology
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Signature of Chairperson
(P. J. DEVARAJU)

**ಈರುಳ್ಳಿಯ (ಏಲಿಯಮ್ ಸೆಪಾ ಎಲ್.) ಬೀಜ ಇಳುವರಿ ಮತ್ತು ಗುಣಮಟ್ಟದ ಮೇಲೆ ಬಾಹ್ಯ
ಮತ್ತು ಜೀವರಸಾಯನಿಕ ಗುಣಲಕ್ಷಣಗಳ ಪರಿಣಾಮದ ಅಧ್ಯಯನ**

ಜ್ಯೋತಿ ನಾರಾಯಣ, ಎ.

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಈರುಳ್ಳಿಯು ಒಂದು ಮುಖ್ಯ ವಾಣಿಜ್ಯ ತರಕಾರಿ ಬೆಳೆಯಾಗಿದ್ದು, ಬೀಜೋತ್ಪಾದನೆಯಲ್ಲಿ ಹೆಚ್ಚಿನ ಮಹತ್ವವನ್ನು ಹೊಂದಿದೆ. ಇತ್ತೀಚಿನ ವರ್ಷಗಳಲ್ಲಿ ನೇರ ಬೀಜ ಬಿತ್ತನೆಯ ಮೂಲಕ ಈರುಳ್ಳಿಯನ್ನು ಬೆಳೆಯಲಾಗುತ್ತಿದೆ, ಆದ್ದರಿಂದ ಉತ್ತಮ ಬೀಜಗಳನ್ನು ಪಡೆಯಲು ಬೀಜಗುಣಮಟ್ಟದ ಪೂರಕ ಲಕ್ಷಣಗಳನ್ನು ಗುರುತಿಸುವುದು ಅನಿವಾರ್ಯವಾಗಿದೆ. ಈ ಅಂಶವನ್ನು ಪರಿಗಣಿಸಿ ಈರುಳ್ಳಿಯಲ್ಲಿ ಬೀಜ ಇಳುವರಿ ಮತ್ತು ಗುಣಮಟ್ಟದ ಮೇಲೆ ಬಾಹ್ಯ ಮತ್ತು ಜೀವರಸಾಯನಿಕ ಗುಣಲಕ್ಷಣಗಳ ಪರಿಣಾಮದ ಬಗ್ಗೆ ಸಂಶೋಧನೆಯನ್ನು ಕೈಗೊಳ್ಳಲಾಯಿತು. ಈ ಅಧ್ಯಯನಕ್ಕೆ ಆಯ್ಕೆ ಮಾಡಿದ ೧೪ ಪ್ರಭೇದಗಳಲ್ಲಿ ೩೬ ವಿವಿಧ ಗುಣಲಕ್ಷಣಗಳು ಅಭ್ಯಸಿಸಲಾಯಿತು. ಎಲ್ಲಾ ಗುಣಲಕ್ಷಣಗಳಲ್ಲೂ ವ್ಯಾಪಕ ಶ್ರೇಣಿಯ ವೈವಿಧ್ಯತೆ ಮತ್ತು ಹೆಚ್ಚಿನ ಅನುವಂಶೀಯತೆ ಕಂಡುಬಂದಿದ್ದು, ಸಸ್ಯಗುಣದರ್ಮಗಳ ಗುಣಾಂಕವೂ ವಂಶಾವಳಿಯ ಗುಣಾಂಕಕ್ಕಿಂತ ಹೆಚ್ಚಾಗಿದೆ. ಸಹಸಂಬಂಧ ಅಧ್ಯಯನವನ್ನು ಪರಿಗಣಿಸಲಾಗಿ ಎಲೆಗಳ ಸಂಖ್ಯೆ, ಗಿಡದ ಎತ್ತರ, ಹೂ ಬಿಡಲು ತೆಗೆದುಕೊಂಡ ದಿನಗಳು, ಹತ್ತು ಈರುಳ್ಳಿಗಡ್ಡೆ ತೂಕ, ಪೂರ್ಣಕರಗಿದ ಸಕ್ಕರೆಯ ಪ್ರಮಾಣ, ಪೂರ್ಣಕರಗಿದ ಪ್ರೋಟೀನ್ ಅಂಶ, α -ಅಮೈಲೇಸ್ ಚಟುವಟಿಕೆಯು ಬೀಜಗುಣಮಟ್ಟದ ಜೊತೆ ಧನಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿವೆ. ಪ್ರತಿಗಿಡದ ಬೀಜ ಇಳುವರಿ, ಬೃಹತ್ ಸಾಂದ್ರತೆ, ಪ್ರತಿ ಹೂಕೊಡೆಯ ಬೀಜಗಳ ಸಂಖ್ಯೆಯು ಬೀಜಗುಣಮಟ್ಟದ ಮೇಲೆ ಋಣಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿವೆ.

ಧನಾತ್ಮಕ ಸಹಸಂಬಂಧ ಹೊಂದಿರುವ ಗುಣಲಕ್ಷಣಗಳನ್ನು ಹೆಚ್ಚಿನ ಅನುವಂಶೀಯತೆ ಮತ್ತು ಅನುವಂಶೀಕ ಮುನ್ನಡೆಯನ್ನು ಹೊಂದಿದ್ದು, ತಳಿ ಅಭಿವೃದ್ಧಿ ವಿಧಾನಗಳಲ್ಲಿ ಪ್ರಾಯೋಗಿಕವಾಗಿ ಬಳಕೆ ಮಾಡಬಹುದು. ಎರಡು ಸಂಕರಣ ತಳಿಗಳಾದ ಆರ್ಕಾ ಲಾಲಿಮಾ ಮತ್ತು ಆರ್ಕಾ ಕೀರ್ತಿಮಾನ್, ನಿಯಂತ್ರಿತ ಹಾಳಾಗುವಿಕೆಯ ನಂತರವೂ ಅತಿಹೆಚ್ಚಿನ ಉತ್ತಮ ಪೋಷಕ, ಮಧ್ಯಮ ಪೋಷಕ ಮತ್ತು ಪ್ರಮಾಣಿತ ಪೋಷಕ ಅನುವಂಶೀಯತೆಯನ್ನು ದಾಖಲಿಸಿದ್ದು, ಅತಿ ಹೆಚ್ಚಿನ ಒಟ್ಟು ಕರಗಿದ ಸಕ್ಕರೆಯ ಪ್ರಮಾಣ, ಒಟ್ಟು ಕರಗಿದ ಪ್ರೋಟೀನ್ ಅಂಶ ಮತ್ತು α -ಅಮೈಲೇಸ್ ಚಟುವಟಿಕೆ ಮತ್ತು ಮೊಳಕೆಯೊಡೆಯುವ ಸಾಮರ್ಥ್ಯವನ್ನು ಹೊಂದಿವೆ.

ಡಿಸೆಂಬರ್, 2016

ಬೆಂಗಳೂರು

ಪ್ರಧಾನ ಸಲಹೆಗಾರರು

(ಪಿ.ಜೆ. ದೇವರಾಜು)



Studies on morphological and biochemical characters attributing seed yield and quality in onion (*Allium cepa* L.)

Jyothi Narayana, A. and P.J. Devaraju

Department of Seed Science and Technology, College of Agriculture, UAS, GKVK, Bengaluru-65



INTRODUCTION

Onion is one of the most important commercial vegetable species belonging to the family *Alliaceae* genus *Allium* and species *cepa*. Besides, it is one of the seven common vegetables grown all around the world. The demand for onion in the world market is gradually increasing owing to its dietary importance. In India it is cultivated in an area of 1203.59 thousand hectares with a production of 19401.69 million tonnes. In the state Karnataka it is cultivated in an area of 136.57 thousand hectares producing about 2065.16 million tonnes. Productivity in Karnataka is about 15.12 tonnes per hectare as against India's average productivity of 17.01 tonnes per hectare.

- ◆ The demand for high quality onion seeds has increased, growers expect high-quality with genetically pure seed.
- ◆ As a result, maintaining seed quality through production and subsequent monitoring of seed from harvest to distribution to farmers is immensely important.
- ◆ However, the literature available on morphological and biochemical characters contributing to seed yield and quality is scanty.
- ◆ The comparative study of improved open pollinated varieties with the parental lines and F_1 hybrids to enhance seed yield and quality of this low volume high value onion seed crop.
- ◆ Further, association of these seed quality attributing and enhancing parameters with plant and seed morphological, physiological and biochemical characters are need of the hour, in order to establish the extent and magnitude of F_1 hybrid vigour over parents, checks and improved OPV's.

Looking into all these factors, it is known that studying seed morphological and biochemical characters attributing to seed quality is great need of the hour.

OBJECTIVES

1. Assessment of seed morphological and biochemical characters influencing seed quality.
2. To identify seed quality attributes associated with hybrid vigour.

MATERIAL AND METHODS

The present field and seed technological experiments were conducted with 14 onion genotypes (*Allium cepa* L.) at Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89 and Department of Seed Science and Technology, COA, UAS, GKVK, Bengaluru-65 respectively as per the recommendations of International Seed Testing Association (ISTA, 2014).

Crop	:Onion (<i>Allium cepa</i> L.)
Season	Seed to bulb - Rabi (2015) Bulb to seed - Summer (2016)
Design	Field-RCBD (III Replications) Lab Experiments-CRD (IV Replications)
Genotypes	G ₁ : Female parent of Arka Lalima G ₂ : Male parent of Arka Lalima G ₃ : Maintainer line of Arka Lalima G ₄ : Arka Lalima F_1 hybrid G ₅ : Female parent of Arka Kirithiman G ₆ : Male parent of Arka Kirithiman G ₇ : Maintainer line of Arka Kirithiman G ₈ : Arka Kirithiman F_1 hybrid G ₉ : Arka Kalyan (OPV) G ₁₀ : Arka Nikethan (OPV) G ₁₁ : Prem (OPV) G ₁₂ : Arka Bindu (OPV) G ₁₃ : Arka Pragathi (OPV) G ₁₄ : Arka Ujwal (OPV)

Acronyms : PCV-Phenotypic Coefficient of Variance; GCV-Genotypic Coefficient of Variance; GA-Genetic Advance; GAM-Genetic Advance Over Mean; GACD-Germination After Controlled Deterioration; SGRW- Seed Growth Rate Weight; MPH-Mid Parent Heterosis; BPH-Better Parent Heterosis; SH-Standard Heterosis

RESULTS

- ◆ Onion genotypes evaluated differed considerably for seed physical, physiological and biochemical quality parameters (Table 1).
- ◆ The correlation coefficients between physical and physiological parameters revealed existence of negative association for seed quality except in case of test weight and true density (0.21 and 0.434 respectively) with positive correlation after controlled deterioration (Table 2) in onion.
- ◆ The biochemical characters exhibited positive correlation with physiological characters. However, after controlled deterioration biochemical parameters recorded negative correlation contributing for Onion seed quality (Table 3).
- ◆ The electrical conductivity of seed leachate was negatively correlated with physiological parameters among Onion genotypes noticeably for seed germination (-0.374) (Table 3.)
- ◆ Seed germination and seedling vigour indices of hybrids (Arka Lalima and Arka Kirithiman) recorded negative heterosis (MPH, BPH and SH). (Table 4.)

Table 1. Estimates of variability parameters for seed quality in onion

Parameters	RANGE		Mean	PCV	GCV	H (BS)	GA	GAM
Test weight (g)	Min.	Max.						
True density (kg/m ³)	2.86	4.16	3.51	11.69	10.91	87.16	0.74	20.98
Germination (%)	454	549	506	5.45	4.2	59.4	33.69	6.66
MSL (cm)	75.50	91.75	84.13	7.19	4.79	44.38	5.53	6.57
SVI-I	13.37	17.9	16.12	8.22	6.23	59.09	1.69	10.01
SVI-II	1114	1506	1358	11.4	9.87	77.85	238.57	17.58
TSS (µg ml ⁻¹)	86.67	104.48	96.85	7.42	4.87	44.12	6.39	6.59
TSP (µg ml ⁻¹)	181.96	336.51	225.55	21.06	17.15	66.35	64.91	28.78
Amylase (µg M ml/min)	273.76	645.66	464.38	27.28	25.25	85.65	223.55	48.14
EC (µ S ppt ⁻¹)	114.21	234.10	138.41	22.23	21.11	90.22	57.18	41.31
EC (µ S ppt ⁻¹)	109.41	267.47	155.11	27.29	25.03	84.14	73.38	47.31

Table 2. Correlation coefficients between physical & physiological parameters for seed quality in onion

Parameters	Germination (%)	SVI-I	SVI-II	GACD (%)
Test weight (g)	-0.235	-0.06	-0.186	0.21
True Density (kg/m ³)	-0.431	-0.501	-0.406	0.434
Seed Size (mm ³)	-0.172	0.083	-0.117	0.004

Table 3. Correlation coefficients between biochemical and physiological parameters for seed quality in onion

Parameters	Germination (%)	SVI-I	SVI-II	GACD (%)
TSS (µg ml ⁻¹)	0.012	0.233	0.026	-0.051
TSP (µg ml ⁻¹)	0.325	0.071	0.223	-0.357
Amylase (µg M ml/min)	0.254	0.276	0.23	-0.328
EC (µ S ppt ⁻¹)	-0.374	-0.239	-0.315	-0.711**

Table 4. Per se performance and heterosis for selected onion hybrids for seed quality attributing traits

Parameters	Mean	MPH		BPH		SH	
		H ₁	H ₂	H ₁	H ₂	H ₁	H ₂
Test weight (g)	3.51	-6.01	-16.86	-11.63	-20.57	-7.31	3.62
True density (kg/m ³)	505.62	6.58	-5.26	5.38	-8.91	11.83	-2.19
No. of seeds/gram	287	1.57	17.85	-4.79	11.27	7.63	-0.87
Seed length (cm)	2.93	0.03	-4.71	-0.23	-6.52	0.88	4.44
Seed width (cm)	2.10	0.45	-12.06	-1.26	-12.35	-4.90	-0.25
Germination (%)	84.13	-2.30	-7.81	-3.34	-8.48	-13.35	-12.81
Root length (cm)	6.85	3.28	-5.54	-0.10	-9.64	-4.89	-0.37
Shoot length (cm)	9.27	-19.24	-4.65	-30.67	-5.85	-9.84	-6.63
MSL (cm)	16.12	-9.49	-5.03	-16.11	-6.16	-7.46	-4.07
SVI-I	1357	-11.52	-12.26	-18.99	-12.69	-19.99	-16.23
MSDW (mg)	1.15	-1.68	-2.12	-2.62	-2.24	-0.31	1.21
SVI-II	97	-3.94	-9.74	-5.84	-10.51	-13.61	-11.75
SGRW	0.10	-1.68	-2.12	-2.62	-2.24	-0.31	1.21
TSS (µg ml ⁻¹)	226	-3.20	23.87	-28.31	14.93	-0.35	1.65
TSP (µg ml ⁻¹)	464	50.69	-30.13	16.49	-37.54	7.89	-35.38
Amylase (µg M ml/min)	138	-14.03	8.96	-17.76	7.71	24.29	12.80
EC (µ S ppt ⁻¹)	155	12.70	11.03	-2.97	3.06	-45.01	-15.40

DISCUSSION

- Genetic variability among physical, physiological and biochemical parameters was meager for seed quality parameters in onion. However, there was an positive association among the characters evaluated (Degevione *et al.*, 2011).
- Weak association of seed germination with physical characters suggest their independent genetic control. While, positive association with biochemical parameters was evident in the present study (Aker *et al.*, 2011).
- Seed germination and vigour indices are the direct measures of seed quality. The negative MPH, BPH and SH in both hybrids might be attributed to existence of negative genetic inheritance for seed quality characters in onion genotypes evaluated.



Fig. 1: Onion seed dimensions recorded using microscope - Nikon SMZ 1000 at 2X magnification

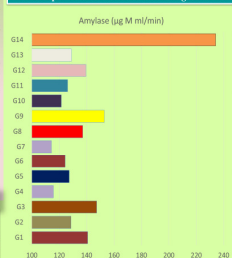


Fig. 2: Amylase activity (µg M ml/min) among genotypes

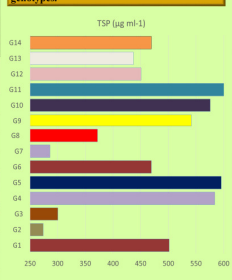


Fig. 3: TSP (µg ml-1) content among genotypes

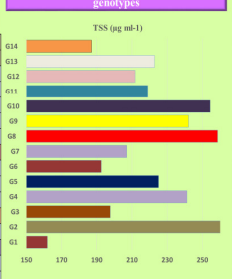


Fig. 4: TSS (µg ml-1) content among genotypes

SUMMARY

- Study revealed existence of positive association between biochemical and physiological parameters contributing for seed quality among onion genotypes studied.
- Seed size, total soluble sugars, total soluble seed protein and seed amylase activity were found to contribute for enhanced seed quality parameters in onion.

ADVISORY COMMITTEE

Chairperson: Dr. P. J. DEVARAJU
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I INTRODUCTION

Onion is one of the most important commercial vegetable species belonging to the family *Alliaceae* genus *Allium* and species *cepa* with a chromosome number of $2n=16$. The genus *Allium* contains about 500 species comprising biennials, perennials and mostly bulbous ones. Onion is thought to have originated from the region comprising of Northwest India, Afghanistan, the Soviet Republic of Tojik, Uzbek and Western Tein Shah. The west Asia region around Mediterranean are the secondary centers of origin.

Onion is an ancient crop having been used in medicine and rituals besides being consumed as food. Onion is said to have diuretic properties, beneficial to digestive tract, good for eyes, used for treatment of chicken pox, the common cold, influenza, measles and rheumatism. The anti-microbial characteristics of *Alliums* is due to the presence of sulfur compounds and the pungency in onion is due to volatile oil 'Allyl propyl disulfide' which varies among cultivars, growing seasons, type of soil, management practices, storage conditions and duration of storage.

Onions not only provide flavor, they also provide important nutrients and health-promoting phytochemicals. Onions are a good source of vitamin C, dietary fiber and folic acid. They also contain calcium, iron and have a high protein quality and low in sodium and contain no fat. Onion is processed in the form of dehydrated products like flakes, powder, pickles and paste. Processed product of onion are in good demand in the local market for defence, fast-food industries and also exported to exotic markets to earn valuable foreign exchange, which is increasing at a rapid rate.

Onion being one among the seven common vegetables grown around the world. The demand for it in the world market is not limited to any climate or any nationality and is probably as widely grown as any other cultivated crop. Onions are found in the markets of the world at all seasons of the year. India is the second largest producer of onion, in the world next to china. In India it is cultivated in an area of 1203.59 t ha, with a production of 19401.69 m.t. Whereas, in Karnataka it is cultivated in an area of 136.57 t ha, producing about 2065.16 m.t. Productivity in Karnataka is about 15.12 t/ha as against India's average productivity of 17.01 t/ha (Indiastat.com, 2015).

Seed is the basic and crucial input in agricultural production. The most important aspect in maintaining continuous supply of high quality seeds to the farmers is to produce genetically pure seeds and to preserve the quality of seeds from harvest to next sowing. Onion seeds are short lived under ambient conditions and exhibit orthodox storage behavior. They can withstand loss of moisture and viability could be extended under low temperature storage conditions. Commercial onion crop is grown either by sowing the seeds directly in the main field or by seedling transplantation. But large scale production of onion is being done by direct seeding. Therefore, the seed quality plays an important role as the crop yield is directly dependent on seedling emergence and establishment. Onion market is characterized by large number of cultivars and strong competition. The demand for high quality onion seeds has increased, thus emphasizing the attention to seed

performance. Growers expect high-quality, genetically pure seed. As a result, seed companies maintain quality control programmes that monitor seed from harvest to purchase. An array of traditional seed quality tests including mechanical, genetic purity, germination, vigour and seed health tests are used allowing seed quality assessment. New developments in computer imaging is also helping in improved, purity and vigour analysis. Advances in molecular genetics are allowing the release of new varieties and hybrids essentially differing in one gene.

In India onion hybrids are being commercialized, the hybrid vigour or yield advantage of F_1 hybrid over open pollinated varieties need to be established. The comparative study of improved open pollinated varieties with the parental lines of F_1 hybrids and also F_1 hybrids seed yield, quality and their association with the plant and seed morphological, physiological and biochemical traits needs to be studied to establish the extent and magnitude of F_1 hybrid vigour over the improved open pollinated varieties.

Although research considers all attributes of seed quality, *e.g.*, genetic, physical, physiological and pathological, literature shows that the physiological component has received more attention with an emphasis on studies regarding reliable procedures for seed vigour evaluation and identification of the relationship between seed vigour and seedling emergence. No vigour test is considered universally accepted for onion seeds but some have been recognized for their effectiveness and research continues moving towards standardization. Onion seeds have a high commercial value and the availability of satisfactory vigour tests for onion seeds is desirable. On the other hand, it is possible to detect effects of seed vigour on subsequent plant growth and performance but a more precise identification of this relationship is needed.

Higher genetic purity is an essential prerequisite for the commercialization of any hybrid seeds. Besides, success of any hybrid technology depends on the availability of quality seed supplied in time at reasonable cost. The genetic purity during multiplication stages is prone to contaminate due to the presence of pollen shedders, out crossing with foreign pollens *etc.*, besides physical admixtures. Thus use of seeds with low genetic purity results in segregation of the traits, lower yields and genetic deterioration of varieties. Genetic purity test is done to verify any deviation from genuineness of the variety during its multiplications. For certification, genetic purity test is compulsory for all foundation and certified hybrid seeds. The traditional Grow out Test (GOT) is done to determine the seed genetic purity test based on morphological markers are time consuming and are environmental dependent.

To overcome this disadvantage, the biochemical markers are being used in many crops. However, repeatability and accuracy of these results on biochemical markers are subject to question. This made a way for the use of DNA molecular markers particularly the co-dominant markers. The SSR markers are of great importance for rapid assessment of hybrid and parental line seed purity (Yashitola *et al.*, 2002). In Onion, a set of SSR markers have been identified to distinguish inbred lines (Tsukazaki, 2009). However, the SSR markers require identification of heterozygosity of the onion hybrids and distinguishing the hybrid in question from its parental lines and other hybrids.

With the development of different tools and techniques, SSR development has been initiated in *Alliums*. To establish a genetic basis for the breeding of Japanese bunching onion, Tsukazaki *et al.* (2008) isolated more than hundreds of SSR clones from size-fractionated genomic DNA libraries. They were highly polymorphic and applicable to the related species such as bulb onion and these can be used for cultivar identification and hybrid purity determination, marker-assisted selection etc. Tsukazaki *et al.* (2007) showed that bunching onion SSRs are very rich sources of highly informative genetic markers by isolating 1796 SSR clones by large-scale sequencing of SSR-enriched genomic DNA libraries.

Molecular marker development in *Allium* was previously restricted due to the giga base genome size and costs of sequencing. Expressed sequence tags (ESTs) from onion that showed significant similarities (70-80 %) to single positions in the rice genome revealed scant collinearity demonstrating that the grasses are not appropriate genomic models for the *Asparagales* this makes it necessary to develop genomic resources for these important plants (Martin *et al.* 2005). Eight novel polymorphic SSR markers developed from an enriched genomic library of garlic (*A. sativum* L.), were used to understand the relationships among 90 garlic accessions (Kyung-Ho *et al.* 2009). Tsukazaki *et al.* (2009) developed an SSR tagged breeding scheme for bunching onion (*A. fistulosum*). Baldwin *et al.* (2010) reported the application of Roche 454 technology to develop sequence resources for population analyses and genetic mapping. So far 170 genomic SSRs, 9 EST-SSRs, 31 Indels and 156 CAPS markers have been developed and tested. Therefore, the present study was conducted to identify a specific SSR marker to discriminate the onion hybrids from its parental lines.

Looking into all these factors, it is known that seed biochemical and morphological characterization is of great need in onion for better seed quality analysis. These characterizations also enable to know the relationship between biochemical and morphological characters with hybrid vigour. Viewed in this context an investigation entitled “Studies on morphological and biochemical characters attributing seed yield and quality in onion (*Allium cepa* L.)” was initiated with the following objectives.

- Assessment of morphological and biochemical characters influencing seed yield and quality.
- To identify the seed quality attributes linked to the hybrid vigour.
- Identification of molecular markers linked to hybridity.

II REVIEW OF LITERATURE

A critical comprehensive review of literature is inevitable for any scientific investigation. The review of literature pertaining to present study involving studies on morphological and biochemical characters attributing seed yield and quality in onion genotypes are presented objective wise.

The present investigation on onion has three broad objectives. As far as possible the review of literature relating to present study is covering onion and where ever the literature is not available on onion other related crops are reviewed and mentioned as under.

2.1 Growth parameter studies.

Mohanty and Prusti (2001) studied behaviour of 12 varieties of onion, during kharif season over two years. The pooled analysis revealed that Arka Niketan, Punjab Red Round, Nasik Red, N 2-4-1, Agrifound Light Red and Arka Pitamber produced small to medium bulb with thinner neck assuring better storage quality. Arka Kalyan recorded the highest yield (21.06 t ha^{-1}) which was on par with Arka Niketan (19.64 t ha^{-1}) and Pusa Madhavi (18.96 t ha^{-1}), while Agrifound Dark Red and N 53 displayed moderately high yield of 18.06 and 17.85 t ha^{-1} respectively. Rabi varieties if grown in kharif season could confer equally good bulb yield as that of kharif varieties. Arka Niketan and Pusa Madhavi with medium bulb, better storage quality and high yield are advocated for commercial cultivation in kharif season.

Pathak (2008) reviewed floral characteristics, male sterility, production of hybrid onion and methods for onion seed production.

Karak and Hazra (2012) studied floral biology of onion by employing 14 genotypes grown as annual crop for seed production, all the eleven floral characters varied significantly among the genotypes. Days required from replanting of the bulb to initiation of scape ranged between 33.12 to 41.67 days. Days required from scape initiation to opening of first flower in the umbel ranged between 26.22 to 32.47 days and it took 26.22 to 32.66 days from opening of first to last flower in the umbel.

2.2 Genetic variability and character association studies.

Mital and Srivastava (1964) worked with two varieties of onion viz, Pushpa red and Early grano. They observed in their experiment that seed yield increases with the increasing number of the flower stalks upto certain limit.

Rathore *et al.* (1980) observed in their experiment that the number of scapes per plant (0.452) and number of flowers per scape (0.325) were directly related to the size of bulb.

The yield of onion seeds was positively related to the sizes of bulbs used for planting. Rathore *et al.* (1980) pointed out that different bulb size had a marked effect on seed yield of onion. The highest yield (13-14 q/ha) was produced by the biggest size bulbs.

In a review paper on onion flowering and seed production Currah (1981) stated that though large bulbs produce more flower stalks, in practice, bulb of moderate size are preferred for seed production.

Borikar *et al.* (1986) in their genetic variability studies for seed vigour parameters in sorghum had reported very high heritability (bs) values for seed germination (%) 987.50, shoot length 992.60, root length (84.9), seed weight (78.2), seed density (77.7), seedling vigour index (94.3) and seedling dry weight (93.2)

Gamiely *et al.* (1990) correlated yields of 'Granex 33' and 'Behairy' onions (*Allium cepa*). Results showed that yields of 'Granex 33' and 'Behairy' closely correlated with the weight of the seeds used to establish the stand. Elemental content was consistently higher in heavier seeds, but elemental concentrations in the seeds were generally negatively related to seed weight, onion growth, and yield. Results showed that combined size-aspiration grading was an effective means of eliminating seed with low-yield potential.

Bhandri and Gupta (1991) opined that phenotypic correlations of grain yield per plant in corainader were highly significant and positive with umbellets per plant, number of effective branches, straw yield per plant, number of primary branches, plant height, number of grains per umbellet and harvest index. Maximum direct contribution to grain yield per plant was made by umbellets per plant, followed by straw yield per plant.

Sidhu *et al.* (1996) conducted an experiment on the seed production potentials of 30 genotypes of onion. They obtained maximum yield from the variety Punjab red round (1.0 t/ha) followed by VL 67 (0.87 t/ha), sel-102-1 (0.87 t/ha) and PW01 (0.87 t/ha).

Cramer (1998) compared open pollinated and hybrid onion varieties at New Mexico and reported that, hybrid varieties out performed open pollinated varieties for plant height and leaf number but not for percentage of seed stalks, pink root incidence, percentage of marketable yield, bulb size and marketable bulb yield.

Villanueva and Havey (2001) evaluated seven inbred lines of onion for seed yield. Males and hybrid differs significantly for seed yield. Significant correlation was observed between mean seed yield per bulb and scape height.

Muhammad *et al.* (2003) concluded that indirect selection based on spikelets per spike, number of grains per spike and grain weight per spike would contribute significantly to develop high yielding wheat genotypes. Path coefficient analysis showed that number of grains per spike had maximum direct effect on grain yield per plant followed by grain weight per spike in wheat. 1000-grain weight had negative direct effect on grain yield per plant.

Kaiser *et al.* (2003) concluded that characters like bulb weight, diameter of bulb and leaf length showed positive effect on yield. They studied effect of eight plant characteristics on the yield through correlation and path analysis. Yield showed positive genotypic and phenotypic correlation with single bulb weight, number of rings per bulb, diameter of bulb and neck, leaf weight and length. Dry matter contents depicted positive correlation with diameter of bulb (0.0807) and neck, and leaf length (0.3390). While bulb weight was positively correlated with number of rings per bulb (0.3253), diameter of bulb (0.6307) and neck (0.6023), number of leaves per plant (0.4006) and leaf length (0.4174).

Singh and Prasad (2006) evaluated 360 lines of coriander (*Coriandrum sativum*), indicating high variability for seed yield (22.82 %), umbels per plant (28.65 %) and seeds per umbel (21.63 %) and low variability for days to 50 % flowering (12.39 %) and umbellets per umbel (13.30 %). High broad sense heritability (91.94 %) and genetic advance (56.55 %) were obtained for umbels per plant and seeds per umbel. Correlation and path coefficient analysis indicated that umbels per plant and branches per plant were the most important traits as they exerted positive direct effect on seed yield.

Aliyu *et al.* (2007) investigated the associations among growth and yield components and their direct and indirect influence on the bulb yield of onion, correlation and path coefficient analysis showed that cured bulb yield had significant positive correlation with plant height (0.826), number of leaves (0.884), bulb diameter (0.809) and bulb weight (0.801).

Haydar *et al.* (2007) reported that among the parameters, plant height, bulb yield and bulb length were found to show high broadsense heritability. Bulb yield per hectare and number of green leaves per plant had high broad sense heritability estimates with high genetic gain. Correlation coefficient and path coefficient analysis in onion indicated that bulb yield had highly positive significant correlation with bulb length and bulb diameter. Bulb diameter also had positive significant association with plant height, fresh weight per bulb and bulb length. The path analysis revealed that plant height, bulb length and bulb diameter are the major components of bulb yield in onion.

Chitra and Rajamani (2009) revealed that seed yield per plant exhibited highly positive significant correlation both at phenotypic and genotypic levels for leaf area, mean leaf area per plant, total chlorophyll, starch content and soluble protein content. The remaining traits viz., total sugar, mean leaf area, leaf area index and relative water content had negatively and non-significantly correlated with dry seed yield per plant both at phenotypic and genotypic levels in glory lily.

Maria *et al.* (2009) revealed that large bulbs produced greater numbers of seed stalks than medium and small ones, and small bulbs produced seed stalks with smaller inflorescences than medium and large ones in onion. They also noticed that the bulb size used in the experiment affected neither the weight of 1000 seeds nor the number of seeds per gram produced from the grown seed stalks. Besides, the length or width of the seeds or the embryos length and width was also unaffected. Further they concluded that the bulb size had no effect on capacity of germination in onion seeds.

Hosamani *et al.* (2010) studied on variability, heritability, genetic advance and inter association between traits in twenty one diverse onion genotypes for bulb yield, yield attributing traits and quality parameters. Results showed that the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were high for yield/ha and average bulb weight. The genetic advance as percentage of mean varied from 10.36 to 47.88 per cent for plant height and average bulb weight respectively. The interrelationship of bulb yield was significantly positive with bulb diameter, bulb neck thickness and average bulb weight. Whereas, in genotypic path coefficient bulb neck thickness had a maximum positive direct effect (3.658) on bulb yield followed by bulb length (2.522), total soluble solids (1.556) and plant height (0.109) and these are good contributors to bulb yield.

Degewione *et al.* (2011) reported that bulb yield was positively and significantly associated with plant height, leaf length, leaf sheath length, leaf sheath diameter, bulb length, bulb diameter, bulb dry weight, biological yield per plant and marketable yield per plant at both phenotypic and genotypic levels. Genotypic path-coefficient analysis revealed that bulb dry weight exerted maximum positive direct effect on bulb yield and also exhibited positive association with bulb yield per plant.

Mallor *et al.* (2011) characterized eighty-six onion landraces from Spain morphologically and physio-chemically. The results evidenced a great variability in all the evaluated traits. Correlations were significant between, firmness ($r = 0.32$), and sucrose content ($r = 0.34$); between fructose and glucose contents ($r = 0.79$); between sucrose content and pungency ($r = 0.57$); between fructose and sucrose contents ($r = -0.22$); and between weight and sucrose content ($r = -0.43$).

Akter *et al.* (2012) suggested that for onion breeding program, emphasis should be given on characters having direct effect on seed yield such as number of filled seeds per umbel, number of flowers per umbel and seed weight.

Ramesh and Sandeep (2012) assessed the nature and magnitude of association among yield and different seed characters. Correlation studies indicated that yield was significantly and positively correlated with all the seed characters under study. Hence direct selection on the basis of seed germination, dry seedling weight, seedling length, seed vigor index-I and seed vigor index-II is reliable for yield improvement in cucumber.

Ashok *et al.* (2013) reported the association among growth characters, yield and bulb quality in onion. They observed that total bulb yield was significantly and positively correlated with bulb fresh weight, vertical bulb diameter, reducing sugars and weight of the bulb at phenotypic and genotypic level.

Dewangan and Sahu (2014) studied the genetic variability, correlation coefficient and path coefficient in onion involving 23 genotypes. High estimates of PCV were recorded in marketable bulb yield, total bulb yield, collar height and average bulb weight. While, GCV were highest in marketable bulb yield and total bulb yield. High heritability coupled with high genetic advance as per cent of mean were observed for total bulb yield, marketable bulb yield, average bulb weight and collar height. Correlation coefficient

analysis revealed that total bulb yield had positive significant correlation with number of leaves per plant, plant height, collar height, polar and equatorial diameter of bulb, average bulb weight and marketable bulb yield. Path coefficient analysis revealed that number of leaves/plant, plant height, leaf thickness, TSS, polar and equatorial diameter of bulb, average bulb weight and marketable bulb yield exhibited positive direct effect on total bulb yield. In addition to this, neck thickness, collar height and leaf length showed negative direct effect on total bulb yield at phenotypic levels and could be utilized as selection criteria in onion improvement programme for Chhattisgarh plains.

Dilruba *et al.* (2014) studied phenotypic and genotypic correlation co-efficient of quantitative characters and character association of aromatic rice. Forty-one aromatic rice germplasm of diverse sources were used to assess the character association and contribution of characters towards grain yield. Experiment revealed that the correlation coefficients of grain yield per hill with 1000-grain weight and harvest index were positive and highly significant (0.529). Spikelet sterility showed highly significant negative correlation (-0.329) with grain yield at genotypic level only. The parameter 1000-grain weight showed the highest positive direct effect on grain yield.

Sharnkumar and Basvegowda (2014) revealed that hundred seed weight (0.749), seed density (0.831), kernel to hull ratio (0.592), root length (0.592), shoot length (0.81), seedling vigor index (0.716), plant height (0.538) at harvest, number of leaves (0.718) at 60 DAS and capitulum diameter (0.804), seed filling percentage (0.615), hundred seed weight (0.546) and seed weight per head (0.781), showed significant positive correlation with commercial kernel yield in sunflower hybrids.

Mohammad *et al.* (2015) revealed that the big bulbs (12 gm) gave larger numbers of umbels per plant than the umbels grown from the medium (8 gm) and small (4 gm) ones. Plant height, number of leaves per plant, length of scape, number of umbels per plant, umble diameter, seed yield per plant, germination percentage and electrical conductivity were positively influenced by bulb size of onion.

2.3 Vigour and heterosis related studies

Falconer (1960) stated that heterosis refers to the superiority of F_1 hybrids and in general to vigour, yield and adoption. The magnitude of heterosis depends on accumulation of favourable dominant alleles in the F_1 population. If parental population differ from each other for more favourable dominant alleles the magnitude of heterosis will also be proportionately higher. This relationship is evidenced in the basic formula for heterosis

$$\text{Heterosis in } F_1 = \sum dy^2$$

Where,

‘d’ is the magnitude of dominance and ‘y’ is difference between the parental populations for allelic frequencies at the locus.

Vadivel *et al.* (1982) evaluated 30 hybrids and 13 parents of *Allium cepa* L. *aggregatum* and the significant positive heterosis over mid parent was observed in fourteen hybrids and over better parent in 12 hybrids, whereas only two hybrids having positive significant heterosis over best parent.

Madalageri (1983) recorded significant positive heterosis over mid parent in six crosses and two crosses have exceeded their better parental value in a (9 × 9) diallel analysis.

Netrapal (1988) reported significant heterosis for plant height over better parent and best parent in (8 × 8) diallel cross.

Divakara (2001) has noticed in his study that positive heterosis in 28 crosses out of 45 F₁'s were for leaf numbers over mid parent, whereas 18 and 11 crosses recorded positive heterosis over better parent and standard check respectively.

Angelica and Julio (2003) reviewed reliable procedures for the accelerated aging and controlled deterioration tests to rank onion seed lots according to their physiological potential. Six seed lots of the cultivars Aurora and Petrolina were evaluated in the laboratory for germination, first count, seedling vigor classification, traditional and saturated salt accelerated aging (41°C / 48 and 72 h), controlled deterioration (24 % of water / 45°C / 24 h) and seedling emergence tests. Seed moisture content after the saturated salt accelerated aging test was lower and uniform, which is considered an important advantage in comparison to the traditional procedure. The saturated salt accelerated aging (41°C / 48 and 72 h) and controlled deterioration (moisture content adjusted to 24 % / 45°C / 24 h) tests were the best procedures to assess the physiological potential of onion seeds, and are indicated for use in quality control programs.

Mumtaz *et al.* (2004) conducted accelerated ageing studies on indigenous onion cultivar "Desi Red" to investigate the vigour and viability in relation to germinability and seed membrane damage. Seeds lost their germinability, vigour and viability progressively with ageing treatment. Germination percentage reduced up to 43 %, radicle length (1.7 cm) and germination speed (6.68) (GSI). Time to get 50 % germination and electrolyte leakage increased significantly ($p < 0.05$) with the ageing time and treatment. While the leaching from viable seeds (control) was negligible. The loss of viability in seeds after ageing appeared related to increased membrane destruction.

Arun *et al.* (2005) studied heterosis in tomato for the total soluble solids. Their findings revealed that crosses CLN-5915-206D4-2-2-0 × CLN-13511E and CLN-1462A × FT-5 recorded highest heterosis over check (Noween).

Shashikanth *et al.* (2007) studied in onion, the extent of heterosis for yield and associated characters, in a set of 30 hybrids produced from a Line x tester mating design involving five lines and six testers of diverse nature maintained in pure form. Appreciable amount of heterobeltosis and standard heterosis was noticed for all the traits studied. The

crosses PBR 139 x AN 184 and PBR 140 x AN 187 recorded high heterosis of 45.31 and 32.83 per cent over better parent and 27.40 and 31.01 per cent over standard check (Arka Kalyan) respectively for marketable bulb yield and were identified as the best hybrid combinations for exploring hybrid vigour. These two crosses also recorded positive and significant heterobeltosis and standard heterosis for other characters studied.

Leena *et al.* (2013) evaluated seeds of sixty hybrids along with ten parents of brinjal for heat tolerance and earliness with national check PH-6 in three replications. Heterosis analysis was carried out for days to 50 % flowering, days to first picking, plant height, number of primary branches per plant, average fruit length (cm), average fruit girth (cm), average fruit weight (g), total number of fruits per plant, number of fruits per plant per picking, marketable fruit yield per plant, total fruit yield per plant and total soluble solids (%). Highest standard heterosis was shown by IBWL x PPC (46.86 %) followed by GL x PPL (46.13 %), MK x IBWL (42.44 %), PPC x WBPF (41.62 %) and WBPF x PR (40.96 %) for total fruit yield per plant in brinjal.

2.4 Molecular marker studies for hybridity conformation and purity testing

Cramer *et al.* (1999) reviewed all the three types of markers viz., 28 morphological, 24 biochemical, and 124 DNA length markers available in onion for characterization.

Martin *et al.* (2008) surveyed genetic variation in a cultivated onion germplasm using simple sequence repeat (SSR) markers and sequenced regions flanking expressed sequence tag (EST) SSRs to develop single-nucleotide polymorphism (SNP) markers. Samples from 89 inbred and open-pollinated (OP) bulb onion populations of wide geographical adaptation and four related *Allium* L. accessions were genotyped with 56 ESTSSR and four genomic SSR markers. Resequencing of 24 marker amplicons revealed additional SNPs in 17 (68 %) and five SNP assays were developed from these, suggesting that resequencing of EST markers can readily provide SNP markers for purity testing of inbreds and other applications in *Allium* genetics.

Muhammad *et al.* (2009) revealed that RAPD and SSR procedures are excellent genomic tools for parentage confirmation and hybridity determination, and would also enhance efficiency of breeding programmes through marker assisted selection. Out of 500 primers surveyed, 3 random and 3 EST based SSR primers were found polymorphic between two cotton parents (FH-883 and FH-631S)

Pallavi *et al.* (2010) suggested that the efficiency of soluble seed protein as marker to distinguish the hybrids from its parental lines is under query due to its low polymorphism and unable to identify heterozygosity because of its dominant nature. While distinguishing the hybrids based on the esterase isozyme banding pattern it could be able to distinguish hybrids from its parental lines and hybrids exhibited bands that are present in both the parental lines thereby confirming the hybridity similar to its parental lines.

Pallavi *et al.* (2010) screened 58 primer pairs in order to identify the specific marker associated with each hybrid and parental lines. Showed that hybrid KBSH-44 could be clearly identified by using ORS 309 and ORS 170, based on the banding pattern resolved on polyacrylamide gel (6 %). The complementary banding pattern of both parents made a way to identify the hybrid. SSR primer ORS 811 found specific to identify KBSH-53 and it amplified allele of size 270 bp in its female parent (CMS-53A) and allele size of 230 bp in its pollen parent (RHA 95-C-1). The hybrid has both the alleles from its parents at 270 and 230 bp.

Solanki *et al.* (2010) studied a total of 24 F₁ plants derived from two crosses viz. Precoz × PL 02 and Sehore 74-3×406 using RAPD and SSR markers for confirming true hybridity. Results showed only five plants as true hybrid

Chethan Kumar *et al.* (2011) used 35 simple sequence repeat (SSR) markers for distinguishing the rice hybrids KRH-2 and DRRH-2 with their parents. Among them, 10 were found to be polymorphic. KRH-2 could be clearly identified by using RM 206, RM 234, and RM 276, Similarly DRRH-2 could be clearly identified by using RM 234, RM 228, and RM 204. Besides, RM 206 for KRH-2 hybrid and RM 228 for DRRH-2 hybrid revealed 100, 93.34 and 91.43 per cent of purity which were validated with the theoretical purity (GOT) per cent of 100, 95 and 90 per cent, respectively.

Sudharani *et al.* (2011) investigated to identify specific marker associated with the maize hybrid DMH-117 and its parental lines, for hybrid purity testing as well as to identify the off-types from the seed lot. The hybrid DHM-117 could be clearly identified by using the SSR loci Umc1600 based on the banding pattern resolved on agarose gel (3 %). The complementary banding pattern of the male and female parents made a way to identify the hybrid. The SSR loci Umc1600 amplified a specific allele size of 180bp in seed parent (BML-6) and 195bp was specific to pollen parent BML-7. These two bands of allele size 180bp and 195bp were found in hybrid DHM-117, there by confirming that this hybrid was produced from the cross combination of BML-6 and BML-7.

Hashemi Petroudi *et al.* (2012) fourteen microsatellite markers for identification of polymorphism between eight rice hybrids and their parental lines. Among them 10 markers were found to be polymorphic. Besides, genetic purity was tested using polymorphic primers which detected off types seeds among F₁ hybrids. Thus microsatellite marker can be used as a reliable tool in DNA profiling and seed purity testing in rice varieties especially the hybrids.

Chinappareddy *et al.* (2013) reviewed the available molecular markers in onion for diversity analysis and varietal identification, linkage and QTL mapping, colour improvement, cytoplasmic male sterility.

III MATERIAL AND METHODS

The details of the methods adopted and materials used during the course of investigation are described in this chapter. The present investigation entitled “Studies on morphological and biochemical characters attributing seed yield and quality in onion” was taken up with three experiments. The study included both field and laboratory experiments in order to determine the association of morphological, physiological and biochemical characters on seed quality.

3.1 Location of the experiment site.

The field experiments were conducted in 8th -block, Division of Vegetable Crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89, geographically located at an elevation of 890 m from mean sea level having latitude of 13°N and longitude of 77° 37' E

Laboratory experiments were conducted in Department of Seed Science & Technology and Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru.

3.2 Climatic conditions

The monthly weather data during the period of investigation and mean over the years recorded at the meteorological observatory of 9th block, vegetable section, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89, are presented in Appendix-III.

3.3 Experimental Details

3.3.1 Experiment I: Assessment of morphological and biochemical characters influencing seed yield and quality.

For the present study, onion seeds were collected from Division of Vegetable Crops, IIHR, Hessaraghatta and they were evaluated for initial seed quality parameters like physical, morphological, physiological and biochemical characters. Further crop was raised at IIHR, Hessaraghatta to determine the plant growth characters contributing to seed yield and quality in onion.

3.3.1.1 Seed material

For the present study 14 genotypes were collected from the Division of Vegetable crops, IIHR, Hessaraghatta.

Sl. No	Genotypes	Source
G₁	: Female parent of Arka Lalima	IIHR
G₂	: Male parent of Arka Lalima	IIHR
G₃	: Maintainer line of Arka Lalima	IIHR
G₄	: Arka Lalima F ₁ hybrid	IIHR
G₅	: Female parent of Arka Kirthiman	IIHR
G₆	: Male parent of Arka Kirthiman	IIHR
G₇	: Maintainer line of Arka Kirthiman	IIHR
G₈	: Arka Kirthiman F ₁ hybrid	IIHR
G₉	: Arka Kalyan (OPV)	IIHR
G₁₀	: Arka Nikethan (OPV)	IIHR
G₁₁	: Prem (OPV)	IIHR
G₁₂	: Arka Bindu (OPV)	IIHR
G₁₃	: Arka Pragathi (OPV)	IIHR
G₁₄	: Arka Ujwal (OPV)	IIHR

Time line of the research activities carried out.

Bulb crop	Date of bulb crop sowing	: 6-7-2015
	Date of transplanting	: 19-8-2015
	Design	: CRD
	Replication	: Three
	Date of harvesting	: 27-11-15
Seed crop	Date of seed crop sowing	: 29-2-2016
	Design	: CRD
	Replication	: Three
	Date of harvesting	: 17-6-16

3.3.1.2 Crop management

The crop was raised following all the agronomic practices as per the package of practices recommended for Onion in the southern dry zone of Karnataka (Anon, 2006).

Nursery management: The seeds were treated with Thiamin @ 2g/kg. Seedlings were raised in well prepared raised beds measuring 2ft × 10ft in 8th –Block, vegetable section, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89. Later seedlings were transplanted to main field in the same block. (Plate. 1, 2)

3.3.1.3 Sampling and collection of data.

Ten plants per plot were randomly selected in each treatment and labeled and following observations were recorded on the tagged plants.

3.3.1.4 Observations recorded:

A. Seed physical characters

- a. Test weight (g)
- b. Bulk density (g/cc)
- c. Number of seeds per gram
- d. Seed length and width (mm)

B. Seed physiological characters

- a. Standard germination (%)
- b. Root length (cm)
- c. Shoot length (cm)
- d. Mean Seedling length (cm)
- e. Seedling vigour index I & II, (ISTA, 2013)
- f. Controlled deterioration test, (ISTA, 2013)

D. Seed biochemical characters

- a. Total soluble seed Protein (Lowry)
- b. Alpha amylase (Sadashivam and Manickam, 1996)
- c. Electrical conductivity of seed leachate. (ISTA.2013)
- d. Total soluble seed sugars. (Sadashivam and Manickam, 1996)

E. Growth and flowering characters

- a. Plant height (cm)
- b. Number of leaves per plant
- c. Length of the flower stalk (cm)
- d. Number of days taken for flower initiation
- e. Number of days taken for 50 % flowering
- f. Number of days taken for completion of flowering
- g. Number of flower stalks
- h. Number of flowers per umbel
- i. Number of seeds per umbel
- j. Number of seeds per flower
- k. Weight of seeds per umbel (g)
- l. Weight of seeds per plant/ seed yield per plant (g)

F. Bulb characters

- a. Bulb polar diameter (cm)
- b. Bulb equatorial diameter (cm)
- c. Number of centers per bulb



Plate 1: Onion seedlings in nursey bed at 40 days ready for transplanting.



Plate 2: General view of onion seed crop in cages.

- d. TSS %
- e. Per cent dry weight (g)
- f. Ten bulb weight (g)

3.3.1.5 Methodology

The procedure followed in recording observation on each trait is being described below

3.3.1.5.1 Test weight (g): One thousand seeds counted manually dried to six per cent moisture content were weighed in eight replicates and mean weight expressed in grams (ISTA, 2013).

3.3.1.5.2 Bulk density (g/cc): Determined by filling an empty 50ml graduated cylinder with the seeds from a height of about 15cm, striking the top level and then weighing the contents (Deshpande *et al*, 1993). The weight of seeds was obtained by subtracting the weight of the cylinder from the weight of the cylinder with seeds. To achieve uniformity in bulk density the graduated cylinder was tapped for the seeds to consolidate. The volume occupied was then noted. The process was replicated thrice and bulk density for each replication was calculated from the equation.

$$\rho_b = \frac{w_s}{v_s}$$

ρ_b - Bulk density in kg/m

w_s - Weight of sample in kg

v_s - Volume occupied by sample in m³

3.3.1.5.3 Number of seeds per gram: Seeds dried to six per cent moisture level were weighed and number of seeds in one gram were counted.

3.3.1.5.4 Seed length and width (mm): Twenty seeds from each genotype were randomly selected to record seed length and width using Nikon SMZ 1000 Imaging microscope. (Plate. 3)

3.3.1.5.5 Standard germination (%): Germination test was conducted in the laboratory using between paper method as per ISTA (2013). Hundred seeds of four replications were placed in between moist non-toxic Kraft paper towels. The rolled towels were incubated in a germinator where a constant temperature of $20 \pm 1^\circ\text{C}$ and 90 % RH was maintained. The germinated seedlings were evaluated on sixth and twelfth day of incubation and the cumulative percentage of germination was expressed based on the normal seedlings.

3.3.1.5.6 Root length (cm): The ten normal seedlings from replication that were selected for measuring the shoot length was used to record the root length. It was from collar region to the tip of primary root and expressed in centimeters.

3.3.1.5.7 Shoot length (cm): Ten normal seedlings in each replication were randomly selected on the final day of seedling evaluation (12th day). Shoot length was measured from the collar region to the attachment of cotyledon and the mean shoot length was expressed in centimeters.

3.3.1.5.8 Mean seedling length (cm): The summation of both root and shoot lengths constituted for mean seedling length and expressed in centimeters.

3.3.1.5.9 Mean seedling dry weight (mg): The ten seedlings used for shoot and root length measurement was used for estimating dry weight. They were dried in a hot air oven maintained at $70 \pm 2^\circ\text{C}$ for 24 hours. After drying and cooling over silica gel for one hour the weight of dry seedlings was recorded and the mean seedling dry weight was calculated and expressed in milligrams.

3.3.1.5.10 Seedling vigour indices: The germination, mean seedling length and dry weight recorded as above were used for the estimation of Seedling vigour Index (SVI) I and II, respectively as per Abdul Baki and Anderson (1973) and expressed as whole number.

$$\text{SVI - I} = \text{Mean seedling length (cm)} \times \text{Germination (\%)} \quad \text{--- (1)}$$

$$\text{SVI - II} = \text{Mean seedling dry weight (mg)} \times \text{Germination (\%)} \quad \text{--- (2)}$$

3.3.1.5.11 Controlled deterioration test: Four replications of 100 seeds each from all the genotypes were initially prepared to raise the moisture levels to 20 % using the formula mentioned below. Once the seeds reached required moisture level, replicates were separately packed into aluminum foil packets and heat sealed. These packets were placed at $7 \pm 2^\circ\text{C}$ for 24 hours for uniform distribution of moisture. Later these seeds with predetermined moisture contents were subjected to deterioration by placing all the replicates into water baths at 45°C for 24 hours $\pm$ 15 minutes. After deterioration seeds were cooled within the packets by placing them under running water for 5 minutes.



Plate 3: Onion seeds length and width measured using NIKON Imaging Microscope at 2X magnification.



Plate. 4: Onion bulbs cut equatorially at maximum diameter to count number of centers per bulb and bulb diameter.

$$\text{Weight of replicate at 20 \% moisture content} = (\text{Initial seed weight}) \times \frac{(\text{100-initial seed moisture content})}{(\text{100-desired seed moisture content})}$$

3.3.1.5.12 Estimation of total soluble seed proteins ($\mu\text{g ml}^{-1}$).

The total soluble seed protein was estimated as per the method prescribed by Lowry *et al.* (1951). The procedure followed is as follows.

a) Preparation of acetone powder

The seed samples were homogenized with chilled acetone in a pestle and mortar. The slurry was filtered immediately under suction pump using Whatman No. 1 filter paper and washed with excess chilled acetone to remove the pigments. The acetone powder was air-dried overnight and stored in sealed containers at 4 °C until further analysis.

b) Preparation of reagents

Solution A: 20g of anhydrous sodium carbonate + 4g of sodium hydroxide were dissolved in 1000ml of water.

Sodium potassium tartarate solution (1.35 %): 1.35g of sodium potassium tartarate was dissolved in water and diluted to 100ml.

Copper sulphate solution (5.5 %): 5.5g of copper sulphate was dissolved in water and diluted to 100 ml.

Solution B: 1.0 ml of 1.35 % sodium potassium tartarate + 0.1 ml of 5.5 % copper sulphate solutions were mixed together.

Solution C: 50 ml of solution 'A' was mixed with 1 ml of solution 'B', just before use.

Folin ciocalteau reagent (FCR): Commercial grade reagent was diluted 1:1 (V/V) before use.

Standard bovine serum albumin (BSA) solution: 2 mg of BSA was dissolved in distilled water and diluted to 10 ml to get a working standard solution containing 200 mg/ml.

c) Sample extraction: One gram of oven-dried (30 ± 2 °C) seed sample (acetone powder) was extracted in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0) for one hour

on a magnetic stirrer at room temperature. The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was used for the estimation of total soluble seed protein content.

d) Estimation of protein: The required quantity (0.1ml) of supernatant was diluted in one ml water. Five ml of solution C was added and mixed. After 10 minutes, 0.5 ml of FCR was added and mixed immediately. After incubation for 30 minutes under dark, the absorbance of the solution was recorded at 660 nm against a reagent blank.

A standard graph was constructed with BSA, with the concentration of 10 to 120 mg. The standard curve was used to estimate the protein content of the sample and expressed in $\mu\text{g ml}^{-1}$ dry weight.

3.3.1.5.13 α -amylase activity ($\mu\text{g M ml/min}$):

The α -amylase activity in developing seeds were estimated according to the method of Sadasivam and Manickam (1996). The enzyme was extracted by grinding 1g of seeds in 1ml of 0.1M phosphate buffer pH 7.2. The ground material was left at 4°C for one hour and centrifuged at 10,000 rpm at 4°C for 15 minutes. The supernatant was used as an enzyme source. To 0.5ml of enzyme extract, 0.5 ml of starch solution was added and incubated at room temperature for 15 minutes. Immediately after incubation, 1 ml of DNS reagent was added boiled for 5 minutes over a water bath and 0.5 ml of 40 per cent sodium potassium tartarate was added to stop the reaction. The solution was cooled at room temperature and 2.5ml of distilled water was added. Blank was prepared without adding starch solution and control without incubation for 15 minutes. The enzyme activity was calculated by measuring OD at 560 nm. α -amylase activity was expressed as μg maltose released/ml/min.

3.3.1.5.14 Electrical conductivity of seed leachate ($\mu\text{S ppt}^{-1}$):

For electrical conductivity (EC) measurements, 25 seeds of four replications were taken at random and washed with acetone followed by distilled water and they were soaked in 25ml of doubled distilled water at a constant temperature of $25 \pm 1^\circ\text{C}$ for a period of 24 hours. Then the steep water (seed leachate) was collected and EC was measured with the help of digital conductivity meter (Model-Digisun DI 9009) and it was expressed in $\mu\text{S ppt}^{-1}$ (ISTA, 2013).

3.3.1.5.15 Total soluble sugars ($\mu\text{g ml}^{-1}$):

The phenol sulphuric acid method for the estimation of sugars

a). Sample preparation : 0.5g seed powder was taken in test tube to which 5 ml of hot 80 % ethanol was added and kept for one hour to extract. Later supernatant was collected and used for estimation.

b). Estimation : 0.1ml of extract was taken into test tubes to which 1ml of 5 % phenol and 5ml of concentrated H₂SO₄ was added. The mixture was allowed to cool at room temperature for 45 minutes. Absorbance was read at 490 nm and values are expressed in µg/ml of extract.

3.3.1.5.16 Plant height (cm): Plant height was recorded at different stages of crop growth i.e. 30, 60 and 90 days after transplanting. It was recorded from the base of the plant to the tip of the leaf and expressed in centimeters.

3.3.1.5.17 Number of leaves per plant: The number of fully grown, green functional leaves were recorded from 10 randomly selected plants at 60 and 90 days after transplanting. The mean of the 10 plants in a genotype was worked out.

3.3.1.5.18 Days to flower initiation: Average number of days taken by representative sample plants to bear at least one completely open umbel.

3.3.1.5.19 Days to 50 % flowering: The number of days taken from the date of sowing to the opening of the petals in 50 % of the plants in each genotype was recorded.

3.3.1.5.20 Days to complete flowering: Average number of days taken by the genotypes to have complete flowering.

3.3.1.5.21 Number of umbels per plant/ bulb: Number of umbels per plant was recorded at the time of harvesting and the average was worked out.

3.3.1.5.22 Number of flowers per umbel: Flowers in individual umbel of the representative plants were counted and recorded.

3.3.1.5.23 Number of seeds per umbel: Seeds from individual umbel were counted and recorded from the representative plants at the time of harvesting.

3.3.1.5.24 Number of seeds per flower: Seeds from individual flowers were counted and recorded from the representative plants at the time of harvesting.

3.3.1.5.25 Weight of seeds per umbel (g): Total weight of seeds from individual umbels were weighed and recorded from the representative plants.

3.3.1.5.26 Seed yield per plant (g): Total seed yield from all the umbels of a particular plant from all the representative plants were weighed and recorded separately.

3.3.1.5.27 Bulb equatorial diameter (cm): The bulb diameter at the middle of the bulb across the polar length was measured with the help of a Vernier calipers and the mean was calculated. (Plate 4)

3.3.1.5.28 Bulb polar diameter (cm): The length between two polar ends was recorded with the help of a Vernier calipers.

3.3.1.5.29 Ten bulb weight (g): 10 competitive bulbs were weighed individually on an electronic balance and the mean value was worked out.

3.3.1.5.30 TSS %: Total soluble solids of bulbs was measured and recorded using brix refractometer, and the average was computed.

3.3.1.5.31 Scape length: It was recorded from the base of the plant to the base of the umbel and expressed in centimeters. (Plate 5)

3.3.1.5.32 Number of centers per bulb: Bulbs were cut equatorially at maximum diameter, to count number of centers.

3.3.2 Experiment II: To identify the seed quality attributes linked to the hybrid vigour.

The magnitude of heterosis was estimated in relation to mid parental and better parental values. They were thus calculated as percentage of increase or decrease of F_1 s over the mid parent value (MP) and better parental value (BP).

a. **Mid Parent Heterosis (MPH)**

$$\text{MPH} = \frac{F_1 - \text{MP}}{\text{MP}} \times 100$$

b. **Better Parent Heterosis (BPH)**

$$\text{BPH} = \frac{F_1 - \text{BP}}{\text{BP}} \times 100$$

Where,

$$\text{MP} = \frac{P_1 + P_2}{2}$$

$$\text{BP} = \frac{P_1 - P_2}{2}$$



Plate 5: Measuring scape length.

MP = Value of mid parent

BP= Value of better parent

F₁ = Value of hybrid

The significance of F₁ heterosis value were tested by comparing them with CD values obtained separately for MP and BP employing the formula given below.

$$\text{CD for MP} = \frac{3}{2} \times \frac{\text{eMSS}}{R} \times \text{'t' value}$$

Where,

eMSS = Error mean sum of squares

r = Number of replications

t = Table 't' value at error degree of freedom

3.3.3 Biometrical analysis

The biometrical analysis of the data (by following the methods explained below) on individual characters was performed using normal statistical package. Statistical methods employed for the analysis are given below.

3.3.3.1 Genetic variability parameters

(i) Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was carried out as per the method suggested by Cochran and Cox (1959). Accordingly, the variance table was set up as given below to test the significant differences among the treatments and replications.

Source	<i>df</i>	Sum of squares	Mean sum of squares	Expected sum of squares
Replication	(r-1)	Rss	Mssr	Mssg/Msse
Treatments (genotypes)	(g-1)	Gss	Mssg	
Error	(r-1)(g-1)	Ess	Msse	

Where,

r = Number of replications

g = Number of genotypes

Rss = Sum of squares due to replication

Gss = Sum of squares due to genotypes

Ess = Sum of squares due to error

Mssr = Mean sum of squares due to replication

Mssg = Mean sum of squares due to genotypes

Msse = Mean sum of squares due to error

σ^2g = Genotypic variance

σ^2e = Phenotypic variance

(ii) Phenotypic and genotypic coefficient of variation (PCV and GCV)

The phenotypic and genotypic coefficient of variation was computed as per Burton and Dewane (1953) for low moisture stress.

$$PCV = \frac{P}{X} \times 100$$

$$GCV = \frac{G}{X} \times 100$$

Where;

P = Phenotypic standard deviation

G = Genotypic standard deviation

X = Grand mean of character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation.

PCV and GCV were classified according to Robinson *et al.* (1949). 0-10 % was considered as low, 10-20 % as moderate and 20 % and above as high.

(iii) Heritability (h^2)

Heritability estimate was calculated using the formula (Hanson *et al.*, 1956) as per cent mean

$$h^2 = \frac{V_g}{V_p} \times 100$$

Where,

h^2 % = Heritability percentage

V_g = Genotypic variance

V_p = Phenotypic variance

Heritability percentage was categorized as follows (Robinson *et al.*, 1949)

0-30 % was considered as low,

30-60 % was considered as moderate

60 % and above as high

(iv) Genetic advance (GA)

Genetic advance was calculated by using formula given by Johnson *et al.* (1955) and was expressed as per cent of mean.

$$\text{Genetic advance (GA)} = h^2 \times \sigma_p \times K$$

Where,

h^2 = Heritability (Broad sense)

σ_p = Phenotypic standard deviation

K = selection differential which is 2.06 at 5 % intensity of selection (Lush, 1949).

3.3.3.2 Estimation of correlation coefficients

To estimate the degree of association between the traits studied, phenotypic correlation was computed by using the formula given by Sundararaj *et al.* (1972).

$$r_{Pxy} = \frac{\text{Cov. } P_{xy}}{\sqrt{P_x \times P_y}}$$

Where,

r_{xy} = correlation co-efficient between X and Y.

COV (X,Y) = Covariance of X and Y.

V(X) and V(Y) = Variances of X and Y.

Phenotypic and Genotypic correlations were calculated using the formula (Singh and Choudary, 1985). The significance of correlation co-efficient was tested by referring to the table value at n-2 d. f. given by Fisher and Yates at 5 % and 1 % significant level.

3.3.3.3 Path coefficient analysis

Path coefficient analysis was carried out as suggested by Wright (1921) and illustrated by Dewey and Lu (1959) in F₂ generations. Standard path coefficients, which are standardized partial regression coefficients, were obtained by solving the following sets of 'P' simultaneous equation by "DOOLITTLE TECHNIQUE" as described by Goulden (1959) using SPAR-1 computer programme as described below.

$$P_{01} + P_{01}r_{12} + \dots + P_{0p}r_{1p} = r_{01}$$

$$P_{01}r_{12} + P_{02} + \dots + P_{0p}r_{2p} = r_{02}$$

$$\cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot$$

$$\cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot$$

$$\cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot$$

$$P_{01}r_{1p} + P_{02}r_{2p} + \dots + P_{0p} = r_{0p}$$

Where , $P_{01}, P_{02}, \dots, P_{0p}$ are the direct path coefficients of the variables, 1,2.....p on the dependent variable 0, $r_{12}, r_{13}, \dots, r_{1p}, \dots, r_{p(p-1)}$ are possible correlation coefficients between various independent variables and $r_{01}, r_{02}, \dots, r_{0p}$ are the correlations between dependent and independent variables.

The indirect effect of i^{th} variable via j^{th} variable is obtained as $P_{0j}r_{ij}$. The contribution of remaining unknown factors was measured as residual factor (r_{ox}), which was calculated as given below.

$$r_{ox} = \sqrt{1 - (P_{01}^2 + 2P_{01}P_{02}r_{12} + 2P_{01}P_{03}r_{13} + \dots + P_{02}^2 + 2P_{02}P_{03}r_{23} + \dots + P_{0p}^2)}$$

3.3.3 Experiment III Identification of molecular markers linked to hybridity

3.3.3.1 Plant materials

Two hybrids Arka lalima and Arka kirthiman with their parents were used for the study.

3.3.3.2 Isolation of genomic DNA

DNA extraction from six genotypes was done using CTAB method from young leaf tissues as per the protocol described by Doyle and Doyle (1990) as below.

- Leaf samples were collected from 21 days old seedlings and stored immediately at -70°C .
- Cetyl trimethyl ammonium bromide (CTAB) extraction (100ml)

CTAB 2 % (w/v)

Tris HCl (pH 8.0) 100 mM

Sodium Chloride 4 M

EDTA 20 mM

(Tris sodium chloride and EDTA were autoclaved and 2 % CTAB was added after autoclaving and preheated before using the buffer).

- 0.2 % β - Mercaptoethanol (added freshly).
- Tris EDTA (TE) buffer

Tris HCl (pH 8.0) :10 mM

EDTA (pH 8.0) :1 mM

(This was dissolved and made up to 100ml, autoclaved and stored at 4 °C)

- 2 % PVP
- Ice cold Isopropanol
- Chloroform: Isoamyl alcohol 24:1 (v/v)
- Sodium acetate (3.0 M, pH 7.0) (pH adjusted using glacial acetic acid)
- Absolute ethanol and 76 % ethanol (stored at -20 °C)
- RNase A: 10 mg/ml (RNase A was dissolved in TE buffer and stored at -20 °C).

Protocol for extraction of genomic DNA

- Leaf samples (approximately 2g) were cut into small bits with sterile scissors and transferred to pre-chilled mortar.
- The leaf tissues were frozen using liquid nitrogen and ground to fine powder.
- The fine powder was allowed to thaw in 10ml of preheated extraction buffer in polypropylene centrifuge tubes and incubated for 30 minutes at 65 °C in water bath with occasional mixing.
- The tubes were removed from the water bath and equal volume of Chloroform: Isoamyl alcohol mixture (24:1 v/v) was added and mixed by inversions for 15 minutes.
- The contents of polypropylene centrifuges tubes were centrifuged at 10,000 rpm for 10 minutes at room temperature.
- The clear aqueous phase was transferred to a new sterile tube and equal volume of ice cold isopropanol was added and mixed gently by inversion and then kept in the freezer at 4 °C for the precipitation of DNA.
- Using blunt end tips, the precipitate was pooled out into a micro-centrifuge tube and air dried after removing the supernatant by brief spin.

- 500 µl of Chloroform: Isoamyl alcohol mixture was added and centrifuged at 10000 rpm for 10 minutes at room temperature.
- Aqueous phase was transferred to another micro-centrifuge tube without disturbing the inner phase.
- To this, 2.5 volume of absolute alcohol and 1/10 volume of sodium acetate were added and kept for incubation overnight.
- It was centrifuged and the supernatant was discarded. To this 500 µl each of 70 % and 100 % ethanol was used subsequently to wash the DNA by centrifugation.
- The alcohol was discarded and DNA was completely air dried and DNA pellet was dissolved in 250 µl of TE and stored at 4 °C.

3.3.3.2 DNA quality assay using agarose gel electrophoresis

Materials

3X loading dye

5 M NaOH	200 µl
95 % formamide (v/v)	95 ml
50 % bromophenol blue (w/v)	50 mg
0.5 % xylene cyanol (w/v)	50 mg

(Dissolved in sterile double distilled water and made up the volume to 100ml)

- 10X Tris Borate EDTA buffer (TBE)

Tris base	107.8 g
Boric acid	55.2 g
EDTA (Na ₂ .2H ₂ O)	9.2 g

(Dissolved in 800 ml of sterile double distilled water and made up to 1000 ml)

- 50X Tris Acetate EDTA buffer (TAE)

Tris base 242 g

Glacial acetic acid 57.1 ml

EDTA ($\text{Na}_2\cdot 2\text{H}_2\text{O}$) (0.5M, pH 8.0) 18.6 g in 100 ml of double distilled water (Dissolved in 800 ml of sterile water and made up to 1000 ml)

- 100bp Ladder

40 μl loading dye

10 μl 100 bp ladder

120 μl sterilized double distilled water

(Total volume made up to 170 μl)

Protocol

- The pyrex gel casting plate's open ends were sealed with cello tape and the comb was placed properly in casting plate kept on a perfectly horizontal platform
- 0.8 % (0.8 g/100 ml) agarose was added to 1X TBE, boiled until the agarose dissolved completely and then allowed to cool. Ethidium bromide (DNA intercalating agent) was added when temperature reached to 55 – 60 °C as the staining agent.
- Then it was poured into the gel mould with combs placed and allowed to solidify. The comb and the cello tape were removed carefully after solidification of the agarose.
- The casted gel was placed in the electrophoresis unit with wells towards the cathode and submerged with 1X TBE to a depth of about 1 cm.

3.3.3.3 Loading the DNA samples

- 1 μl of the crude DNA sample was pipetted onto a parafilm and mixed well with 3 μl of 3X loading dye by pipetting up and down gently several times.
- The mixed contents were transferred to the wells of agarose gel placed inside the 1X TBE buffer.

3.3.3.4 Quantification of DNA

The genomic DNA was quantified spectrophotometrically both at 260 nm and 280 nm wavelengths. The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg of double stranded DNA. A pure sample of DNA shows the ratio of OD 260/280 as 1.8. Ratios less than 1.8 indicate contamination in the isolation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the isolation.

3.3.3.5 Normalization of the DNA concentration

Normalization of the DNA concentration was done to bring all the DNA concentrations to a relatively equal level (25 ng/µl) by appropriate dilutions for PCR reaction. Dilution was done with double distilled sterile water.

3.3.3.6 Selection of primers:

Ten SSR primer pairs which gave clear and reproducible bands in earlier studies were selected for amplification of samples (Tsukazaki *et al.*, 2008).

3.3.3.7 Genic SSR markers used

SL. NO	PRIMER	SEQUENCE	NUCLEOTIDE LENGTH (bp)	ANNEALING TEMP. (°C)
1	AFA06A08_F	CCTCAGGAGAGGGGTATTTTGGTT	24	52
2	AFA06A08_R	CTTGGGAAAGGCTTCTCTTGAGGT	24	52
3	AFAA01D09_F	GCCATAATGGAGCATCCAAAACT	24	50
4	AFAA01D09_R	AAAGCAACTTGCATCGTACGTCAT	24	50
5	AFB03G07_F	GGCTGTTTGAAGCCCTTCTT	21	50
6	AFB03G07_R	ATGCGTGTATGTGTGCGTGTG	21	50
7	AFB23B03_F	CCTATTCGCTTCCACCATGATAGC	24	50
8	AFB23B03_R	AAAGGTGACGTGAAGGCTACTTCG	24	50
9	AFC12A06_F	ACGACATATTTTGATACGGTTTTGAGC	27	50
10	AFC12A06_R	GAATAACTTTAGCATAACTTCGACGCATT	29	50
11	AFRA07E10_F	CAGAACTAGACGGTTTGGGAGGAA	24	50
12	AFRA07E10_R	AGTCATATTTTGAAGCTCGCAGG	24	50
13	AFRT01F02_F	ACGAGGCATAGGTGGATGAGGTTA	24	50
14	AFRT01F02_R	CAGCCAACTCGTCATAGGAACGATA	25	52
15	AFRT01F06_F	CCTTTGTCCCTCTCATCATA	21	52
16	AFRT01F06_R	ACATGCAAATGCACATACACA	21	50
17	AFS039_F	CGGGTAATAACGGATATCATAAACA	25	50
18	AFS039_R	CAGTTGTTACATGTGGTATCAGAGC	25	50
19	AFS105_F	ACTTTCGTTGCAAACATTTACACACAC	27	50
20	AFS105_R	GTTGGAGTTTACTTCCCCAAATGAAG	25	50

PCR specifications:**PCR mixture:**

Template DNA: 12-20 ng

Primer: 0.25 μ M

dNTPs: 200 μ M

1X TBE buffer

1 unit *Taq* polymerase

PCR amplification:

Denaturation: 5 min 94 $^{\circ}$ C for 35 cycles

Annealing : 1 min 40-65 $^{\circ}$ C (depending on the melting temperature of primers) and 1 min of elongation at 72 $^{\circ}$ C followed by final incubation at 72 $^{\circ}$ C for 5min for prime extension; gel electrophoresis at 120 V

3.3.3.8 Gel electrophoresis

Agarose gel was prepared using electrophoresis grade agarose in a volume of electrophoresis buffer (1x TAE) sufficient for constructing a gel (300 ml for 18 x 30 cm gel). Ethidium bromide was added at concentration of 0.5 μ g/ml of gel. The gel was allowed to set fully before removing the comb and loading the samples. Five μ l of loading dye was added to 20 μ l of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between wells. A voltage of 1-5 v/cm was given for a time period of three hours for separation of PCR fragments. After electrophoresis gel was viewed under UV light and the DNA banding pattern was recorded directly or photographed.

3.3.3.9 Statistical analysis

The data obtained from the experiment were statistically analyzed by using appropriate ANOVA by WINDOWSTAT version 9.1 from INDOSTAT services, Hyderabad licenced to Agriculture Knowledge Management Unit, GKVK, UAS, Bengaluru. The critical differences between the treatments were worked out at five per cent significance (Snedecor and Cochran, 1967).

IV EXPERIMENTAL RESULTS

The present study entitled “Studies on morphological and biochemical characters attributing seed yield and quality in onion”, was conducted in 8th -block, Division of vegetable crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89 and Laboratory experiments were conducted in Department of Seed Science & Technology. The results pertaining to the field and laboratory experiments are presented in detail.

4.1 Initial seed quality parameters

The initial seed quality parameters *viz.*, germination, seedling length, seedling dry weight, seedling vigour index based on seedling length and seedling dry weight and electrical conductivity were recorded and presented in Table 4.1.

Significant differences were found among the genotypes. The highest germination was recorded in G₁₀ (90.7 %) followed by G₈ (89.7 %), G₇ (88.7 %), G₄ (86.7 %) and least was in G₉ (76 %), highest seedling length was recorded in G₅ (18.25 cm) followed by G₁₃ (17.57 cm) and least was in G₁₄ (13.88 cm). Seedling dry weight was recorded highest in G₈ (1.18 mg) followed by G₂ (1.17 mg) and lowest in G₁₀ (1.13 mg).

Seedling vigour index-I was highest in G₁₃ (1506) followed by G₈ (1479) and least observed in G₁₂ (1157). Whereas, seedling vigour index-II was highest in G₈ (105) followed by G₇ (102) and G₁₀ (102) and least in G₉ (88). The electrical conductivity analysed among the genotypes G₁ (112.75 $\mu\text{S ppt}^{-1}$) recorded lowest followed by G₅ (117.67 $\mu\text{S ppt}^{-1}$) and G₉ recorded highest electrical conductivity value (254.13 $\mu\text{S ppt}^{-1}$).

4.2 Analysis of variance (ANOVA).

The mean sum of squares due to genotypes for different characters are presented in Table 4.2. From the data it is evident that the 14 genotypes differed significantly for all the characters studied.

4.3. Estimation of components of genetic variability.

4.3.1 Estimation of components of genetic variability for plant characters in onion.

The range, mean and coefficient of variation for different plant characters are presented in table 4.3. Considerable range of variation was observed in number of seeds per plant (642.0-3755.35), ten bulb weight (118.33-453.33 g), number of seeds per umbel (180-835.83), number of flowers per umbel (196.65-351), days to 50 per cent flowering (51.30-71.30).

The coefficient of variability gave an indication of the relative corresponding to the magnitude of the mean. The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the plant characters.

Table 4.1: Initial seed quality parameters as influenced by different onion genotypes.

Sl. No	Genotypes	Germination (%)	Seedling length (cm)	Mean seedling dry weight (mg)	SVI-I	SVI-II	EC (μ S ppt ⁻¹)
1	G ₁	81.3	14.64	1.14	1190	93	112
2	G ₂	86.7	16.79	1.17	1455	101	151
3	G ₃	79.3	15.63	1.17	1230	92	135
4	G ₄	86.7	16.63	1.17	1441	101	147
5	G ₅	81.0	18.25	1.16	1477	94	117
6	G ₆	85.3	16.25	1.17	1386	100	137
7	G ₇	88.7	17.06	1.15	1511	102	157
8	G ₈	89.7	16.50	1.18	1479	105	144
9	G ₉	76.0	15.63	1.16	1190	88	254
10	G ₁₀	90.7	16.02	1.13	1453	102	132
11	G ₁₁	85.7	16.90	1.17	1446	100	206
12	G ₁₂	76.3	15.23	1.16	1157	88	146
13	G ₁₃	85.7	17.57	1.17	1506	100	145
14	G ₁₄	83.7	13.88	1.16	1161	97	165
	Mean	84.1	16.21	1.16	1363	97	153
	SEm$\pm$	2.69	0.54	0.02	46.13	3.82	7.66
	C.D (P=0.05)	7.81	1.57	0.06	133.65	11.07	22.20
	CV (%)	5.55	5.78	3.04	5.86	6.79	8.62

G₁ : Female parent of Arka Lalima

G₂ : Male parent of Arka Lalima

G₃ : Maintainer line of Arka Lalima

G₄ : Arka Lalima F₁ hybrid

G₅ : Female parent of Arka Kirthiman

G₆ : Male parent of Arka Kirthiman

G₇ : Maintainer line of Arka Kirthiman

G₈ : Arka Kirthiman F₁ hybrid

G₉ : Arka Kalyan (OPV)

G₁₀ : Arka Nikethan (OPV)

G₁₁ : Prem (OPV)

G₁₂ : Arka Bindu (OPV)

G₁₃ : Arka Pragathi (OPV)

G₁₄ : Arka Ujwal (OPV)

Table 4.2: Analysis of variance (ANOVA) of all studied characters.

Sl. No.	Characters	Source of variation	Degrees of freedom	Mean sum of squares
1	Plant height (cm)	Replication	2	12.4049
		Treatments	13	71.0335
		Error	26	15.6809
2	Number of leaves	Replication	2	0.1422
		Treatments	13	6.3384
		Error	26	0.4929
3	Scape length (cm)	Replication	2	2.1189
		Treatments	13	82.2010
		Error	26	16.3951
4	Days to flower initiation	Replication	2	0.4582
		Treatments	13	57.0428
		Error	26	3.3697
5	Days to 50 % flowering	Replication	2	11.8325
		Treatments	13	97.9829
		Error	26	4.7737
6	Days to completion of flowering	Replication	2	16.6245
		Treatments	13	154.9295
		Error	26	6.6594
7	Number of umbels per plant	Replication	2	0.1693
		Treatments	13	1.6905
		Error	26	0.0651
8	No of flowers per umbel	Replication	2	268.8072
		Treatments	13	6066.2345
		Error	26	90.5218
9	No of seeds per umbel	Replication	2	434.2960
		Treatments	13	144515.3020
		Error	26	215.2977
10	Number of seeds per flower	Replication	2	0.0318
		Treatments	13	1.6307
		Error	26	0.0129
11	Number of seeds per plant	Replication	2	4751.2000
		Treatments	13	2680136.8751
		Error	26	3044.5159

Sl. No.	Characters	Source of variation	Degrees of freedom	Mean sum of squares
12	Seed yield per plant (g)	Replication	2	0.0472
		Treatments	13	28.2393
		Error	26	0.0356
13	Ten bulb weight (g)	Replication	2	357.7381
		Treatments	13	32422.3901
		Error	26	1021.8406
14	Total soluble solids (brix %)	Replication	2	.6238
		Treatments	13	11.6791
		Error	26	0.3636
15	Equatorial diameter (cm)	Replication	2	0.1156
		Treatments	13	2.7747
		Error	26	0.1447
16	Polar diameter (cm)	Replication	2	0.1463
		Treatments	13	2.5082
		Error	26	0.0989
17	Number of centers per bulb	Replication	2	1.3027
		Treatments	13	2.7348
		Error	26	0.1989
18	Per cent dry weight (g)	Replication	2	0.0828
		Treatments	13	1.1640
		Error	26	0.1825
19	Test weight (g) (1000 seeds)	Replication	2	0.0062
		Treatments	13	0.4622
		Error	26	0.0216
20	Bulk density (g/cc)	Replication	2	846.4891
		Treatments	13	1658.9593
		Error	26	307.8213
21	Number of seeds per gram	Replication	2	30.3095
		Treatments	13	3403.8827
		Error	26	22.0531
22	Seed length (mm)	Replication	2	0.0281
		Treatments	13	0.0548
		Error	26	0.0103

23	Seed width (mm)	Replication	2	0.0144
		Treatments	13	0.0338
		Error	26	0.0053
24	Total soluble sugars ($\mu\text{g ml}^{-1}$)	Replication	2	172.2755
		Treatments	13	6801.5993
		Error	26	347.4832
25	Total soluble proteins ($\mu\text{g ml}^{-1}$)	Replication	2	339.6983
		Treatments	13	39618.4784
		Error	26	1017.4880
26	α -amylase activity ($\mu\text{g M ml/min}$)	Replication	2	132.4520
		Treatments	13	2482.9671
		Error	26	96.2081
27	Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	Replication	2	7.0194
		Treatments	13	1655.9917
		Error	26	143.6785
28	Germination (%)	Replication	2	20.7380
		Treatments	13	65.0695
		Error	26	20.3278
29	Shoot length (cm)	Replication	2	0.0586
		Treatments	13	1.4061
		Error	26	0.2584
30	Root length (cm)	Replication	2	0.5292
		Treatments	13	1.3589
		Error	26	0.1129
31	Seedling length (cm)	Replication	2	0.7720
		Treatments	13	4.6029
		Error	26	0.4193
32	Mean seedling dry weight (mg)	Replication	2	0.0023
		Treatments	13	0.0009
		Error	26	0.0008
33	Seedling vigour index-I	Replication	2	2344.4380
		Treatments	13	88762.2328
		Error	26	6037.7571
34	Seedling vigour index-II	Replication	2	3.7788
		Treatments	13	133.0451
		35Error	26	32.3625
35	Germination after controlled deterioration (%)	Replication	2	3.3774
		Treatments	13	136.7603
		Error	26	12.4527

Table 4.3: Estimation of components of genetic variability for plant characters in onion.

Growth characters	Mean	Range	GCV	PCV
Plant height (cm)	62.1	52.5 - 67.7	6.91	9.40
Number of leaves	11.3	8.0 - 13.5	12.32	13.74
Scape length (cm)	59.8	48.16- 67.5	7.82	10.34
Days to flower initiation	43.78	36.06 - 51.30	9.66	10.53
Days to 50 % flowering	63.8	51.3 - 71.3	8.73	9.37
Days to completion of flowering	74.9	58.4 - 86.2	9.38	9.99
Number of umbels per plant	3.4	2.2 - 4.5	21.22	22.46
No of flowers per umbel	281.6	196.6 - 351.0	15.84	16.20
No of seeds per umbel	412.7	180 - 835.8	53.13	53.25
Number of seeds per flower	3.2	2.2 - 4.5	22.52	22.79
Number of seeds per plant	1562.8	642.0 - 3755.3	60.44	60.54
Seed yield per plant (g)	5.25	2.15 - 10.69	58.40	58.51
Ten bulb weight (g)	313.4	118.3 - 453.3	32.63	34.19
Total soluble solids (brix %)	11.7	9.3 - 15.8	16.51	17.28
Equatorial diameter (cm)	4.7	3.1 - 6.2	19.51	21.06
Polar diameter (cm)	4.2	2.4 - 5.8	21.21	22.48
Number of centers per bulb	3.8	1.7 - 5.6	23.92	26.58
Per cent dry weight (g)	8.7	7.8 - 9.6	6.53	8.16

GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation;

The plant characters such as number of seeds per plant had highest GCV (60.44) and 60.54 (PCV) followed by seed yield per plant (58.40 GCV and 58.51 PCV), number of seeds per umbel (53.13 GCV and 53.25 PCV), ten bulb weight (32.63 GCV and 34.19 PCV) and number of centers per bulb (23.92 GCV and 26.58 PCV) respectively. Moderate GCV and PCV was observed in number of seeds per flower (22.52 GCV and 22.79 PCV) followed by number of umbels per plant (21.22 GCV and 22.46 PCV), polar diameter (21.21 GCV and 22.48 PCV), respectively and all other characters recorded lowest GCV and PCV.

4.3.2 Estimation genetic variability for seed characters of onion genotypes.

The range, mean and coefficient of variation for different seed characters are presented in table 4.4. Considerable range of variation was observed in seedling vigour index-I (1149.9-1652.96), total soluble proteins (273.46-645.65 $\mu\text{g ml}^{-1}$), total soluble sugars (161.95-369.48 $\mu\text{g ml}^{-1}$), and electrical conductivity (109.41-206.66 $\mu\text{S ppt}^{-1}$).

The coefficient of variability gave an indication of the relative corresponding to the magnitude of the mean. The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the seed characters.

Table 4.4: Estimation of components of genetic variability for seed characters in onion.

Seed characters	Mean	Range	GCV	PCV
Test weight (g) (1000 seeds)	3.5	2.2 - 4.1	10.90	11.68
Bulk density (g/cc)	505.61	453.76 - 549.05	4.19	5.44
Number of seeds per gram	287.4	241.6 - 353.6	11.67	11.79
Seed length (mm)	2.9	2.6 - 3.1	4.15	5.42
Seed width (mm)	2.1	1.9 - 2.2	4.63	5.79
Total soluble sugars ($\mu\text{g ml}^{-1}$)	230.7	161.9 - 369.4	20.09	21.65
Total soluble proteins ($\mu\text{g ml}^{-1}$)	472.8	273.4 - 645.6	23.99	24.92
α -amylase activity ($\mu\text{g M ml/min}$)	141.37	114.21 - 233.87	19.95	21.12
Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	144.6	109.4 - 206.6	15.52	17.59
Germination (%)	85.9	76.3 - 92.0	4.49	6.90
Shoot length (cm)	9.5	7.8 - 10.2	6.50	8.42
Root length (cm)	6.9	5.3 - 7.6	9.30	10.48
Seedling length (cm)	16.4	13.7 - 17.6	7.18	8.19
Mean seedling dry weight (mg)	1.1657	1.1404 - 1.2101	0.61	2.52
Seedling vigour index-I	1415	1149 - 1652	11.72	12.94
Seedling vigour index-II	100	87 - 111	5.77	8.10
Germination after controlled deterioration (%)	55.9	40 - 65	11.49	13.11

GCV: Genotypic Coefficient of Variation

PCV: Phenotypic Coefficient of Variation

The seed character total soluble proteins had highest (23.99 GCV and 24.92 PCV) followed by total soluble sugars (20.09 GCV and 21.65 PCV), α -amylase activity (19.95 GCV and 21.12 PCV), electrical conductivity (15.52 GCV and 17.59 PCV) and seedling vigour index-I (11.72 GCV and 12.94 PCV) respectively. Moderate GCV and PCV was observed in number of seeds per gram (11.67 GCV and 11.79 PCV) followed by germination after controlled deterioration (11.49 GCV and 13.11 PCV), respectively and all other characters recorded lowest GCV and PCV.

4.4 Estimation of heritability (broad sense) and genetic advances (% mean).

4.4.1 Estimation of heritability (broad sense) and genetic advances (% mean) for plant characters.

The estimates of heritability (broad sense) and genetic advance for all plant characters are presented in Table 4.5.

Table 4.5: Estimation of heritability (broad sense) and genetic advances (% of mean) of plant characters influenced by different genotypes in onion.

Plant characters	Heritability (h^2)	Genetic advance (%) mean
Plant height (cm)	54.1	10.46
Number of leaves	79.8	22.68
Scape length (cm)	57.2	12.19
Days to flower initiation	84.2	18.25
Days to 50 % flowering	86.7	16.74
Days to completion of flowering	88.1	18.14
Number of umbels per plant	89.3	41.3
No of flowers per umbel	95.7	31.9
No of seeds per umbel	99.6	109.22
Number of seeds per flower	97.6	45.89
Number of seeds per plant	99.7	124.30
Seed yield per plant (g)	99.6	120.08
Ten bulb weight (g)	91.1	64.17
Total soluble solids (brix %)	91.2	32.48
Equatorial diameter (cm)	85.8	37.23
Polar diameter (cm)	89.0	41.24
Number of centers per bulb	81.0	44.34
Per cent dry weight (g)	64.2	10.79

High estimates of heritability (> 60 %) was recorded in number of seeds per plant (99.7 %), number of seeds per umbel (99.6 %), seed yield per plant (99.6 %), number of seeds per flower (97.6 %), number of flowers per umbel (95.7 %), Total soluble sugars (91.2 %), ten bulb weight (91.1 %), number of umbels per plant (89.3 %).

Moderate heritability was recorded in scape length (57.2 %) and plant height (54.1 %). Highest genetic advance under selection pressure was recorded for number of seeds per plant (124.30 %), seed yield per plant (120.08 %), number of seeds per umbel (109.22 %), ten bulb weight (64.17 %), number of seeds per flower (45.89 %), number of centers per bulb (44.34 %), and polar diameter (41.24 %). Moderate magnitude of genetic advance was observed in plant characters namely days to flower initiation (18.25 %), days to completion of flowering (18.14 %), days to 50 per cent flowering (16.74 %), scape length (12.19 %), and plant height (10.46 %). Whereas none of the characters recorded low magnitude of genetic advance.

High heritability coupled with high genetic advance was observed for number of seeds per plant, number of seeds per umbel, seed yield per plant, number of umbels per plant, number of centers per bulb and polar diameter.

4.4.2 Estimation of heritability (broad sense) and genetic advances (% mean) seed characters.

The estimates of heritability (broad sense) and genetic advance for all seed characters are presented in Table 4.6.

High estimates of heritability (> 60 %) was recorded in number of seeds per gram (98.10 %), total soluble proteins (92.7 %), α amylase activity (89.2 %), total soluble sugars (86.1 %), test weight (87.2 %), seedling vigour index (82 %), root length (78.6 %), electrical conductivity (77.8 %).

Moderate heritability was recorded in shoot length (59.7 %), seedling dry weight (56.7 %), seedling vigour index-II (50.9 %), bulk density (59.4 %) seed length (58.8 %) and germination (42.3 %). Low heritability estimate was found for seedling dry weight (5.9 %).

Highest genetic advance under selection pressure recorded for total soluble proteins (47.57 %), α amylase activity (38.81 %), total soluble sugars (38.41 %), electrical conductivity (28.20 %), number of seeds per gram (23.82 %), seedling vigour index-I (21.88 %), test weight (20.98 %) and germination after controlled deterioration (20.77). Moderate magnitude of genetic advance was observed in seed characters namely root length (16.99 %), seedling length (12.97 %) and shoot length (10.35 %).

Whereas, very low genetic advance was observed in seedling vigour index-II (8.49 %), seed width (7.63 %), seed length (6.57 %), germination percentage (36.02 %) and seedling dry weight (0.30 %). High heritability coupled with high genetic advance was

observed for total soluble proteins, total soluble sugars, α amylase activity, number of seeds per gram and test weight.

Table 4.6: Estimation of heritability (broad sense) and genetic advances (% of mean) of seed characters influenced by different genotypes in onion.

Seed characters	Heritability (h ²)	Genetic advance (%) mean
Test weight (g) (1000 seeds)	87.2	20.98
Bulk density (g/cc)	59.4	6.66
Number of seeds per gram	98.10	23.82
Seed length (mm)	58.8	6.57
Seed width (mm)	64.0	7.63
Total soluble sugars ($\mu\text{g ml}^{-1}$)	86.1	38.41
Total soluble proteins ($\mu\text{g ml}^{-1}$)	92.7	47.57
α -amylase activity ($\mu\text{g M ml/min}$)	89.2	38.81
Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	77.8	28.20
Germination %	42.3	6.02
Shoot length (cm)	59.7	10.35
Root length (cm)	78.6	16.99
Seedling length (cm)	76.9	12.97
Seedling dry weight (mg)	5.90	0.30
Seedling vigour index-I	82.0	21.88
Seedling vigour index-II	50.9	8.49
Germination after controlled deterioration (%)	76.9	20.77

4.5 Association of characters

4.5.1 Correlation studies.

4.5.1.1 Estimation phenotypic (p) correlation among different plant characters

Correlation studies were conducted to understand the relationship between various plant morphological, flowering, and bulb characters on seed quality parameters. The data pertaining to phenotypic correlation coefficients for all possible pairs involving 18 characters are presented in Table 4.7 and the details are presented below:

The data revealed that seed germination was positive and highly significantly correlated with seedling vigour index-I (0.819) and seedling vigour index-II (0.822). It was positively correlated with number of leaves (0.135), days to flower initiation (0.254), number of umbels per plant (0.228), seed yield per plant (0.243), number of seeds per plant (0.284) and ten bulb weight (0.213). Seed germination was negatively correlated with number of centers per bulb (-0.232).

Seedling vigour index-I revealed negative correlation with number of centers per bulb (-0.144) and positive significant correlation with number of leaves (0.328), days to flower initiation (0.33) and equatorial bulb diameter (0.355). It showed highly significant positive correlation with number of umbels per plant (0.463).

The characters such as number of leaves (0.219), days to flower initiation (0.291), number of umbels per plant (0.281), number of seeds per plant (0.259), ten bulb weight (0.301) and polar bulb diameter (0.252) showed positive correlation with seedling vigour index-II. It showed negative correlation with number of centres per bulb (-0.258).

Polar bulb diameter showed highly significant positive correlation with plant height (0.579), number of leaves (0.676), number of umbels per plant (0.734), ten bulb weight (0.577), equatorial diameter (0.849) and significant positive correlation with scape length (0.319). It revealed significant negative correlation with number of seeds per umbel (-0.517), number of flowers per umbel (-0.395), seed yield per plant (-0.395) and total soluble solids (-0.525). Similar trend was followed by equatorial diameter.

Characters like number of flowers per umbel (0.648), number of seeds per umbel (0.559), number of seeds per plant (0.478), and seed yield per plant (0.491) showed highly significant positive correlation with total soluble solids in bulbs. It showed significant negative correlation with number of leaves (-0.322) and highly significant negative correlation with number of umbels per plant (-0.475), equatorial diameter (-0.512), polar diameter (-0.525) and ten bulb weight (-0.466).

Ten bulb weight showed highly significant positive correlation with number of umbels per plant (0.417), equatorial bulb diameter (0.549), polar bulb diameter (0.577), seedling vigour index-I (0.397) highly significant negative correlation with total soluble solids (-0.466). It showed negative correlation with seed yield per plant (-0.204).

Seed yield per plant revealed highly significant positive correlation with number of seeds per umbel (0.916), number of seeds per plant (0.961), and total soluble solids (0.491). It showed significant positive correlation with days to flower initiation (0.313) and highly significant negative correlation with number of leaves (-0.473), equatorial diameter (-0.396) and polar diameter (-0.447).

Characters like plant height (0.419), number of leaves (0.530), days to flower initiation (0.517), number of umbels per plant (0.611) showed highly significant positive correlation with scape length while number of flowers per umbel (-0.151), number of seeds per umbel (-0.205), number of seeds per plant (-0.118), number of centers per bulb (-0.173) showed negative correlation.

Table 4.7: Phenotypic correlation coefficients between plant growth, flowering, bulb and seed quality characters of onion genotypes.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈
X ₁	1	0.641**	0.419**	0.418**	0.440**	0.131	-0.191	-0.208	-0.171	0.118	-0.171	0.416**	0.579**	-0.382*	-0.132	0.079	0.115	0.137
X ₂		1	0.530**	0.309*	0.720**	-0.003	-0.418**	-0.387*	-0.473**	0.249	-0.322*	0.604**	0.676**	-0.043	-0.054	0.135	0.328*	0.219
X ₃			1	0.517**	0.611**	-0.151	-0.205	-0.118	-0.108	0.069	-0.218	0.035	0.319*	-0.173	0.041	-0.047	0.014	-0.01
X ₄				1	0.445**	-0.315*	0.057	0.237	0.313*	0.377*	-0.215	0.139	0.284	-0.409**	0.082	0.254	0.330*	0.291
X ₅					1	-0.182	-0.432**	-0.248	-0.332*	0.417**	-0.475**	0.611**	0.734**	-0.172	0.16	0.228	0.463**	0.281
X ₆						1	0.23	0.11	0.138	-0.156	0.648**	-0.077	-0.092	-0.16	-0.603**	0.127	-0.004	0.154
X ₇							1	0.940**	0.916**	-0.364*	0.559**	-0.394**	-0.517**	0.029	-0.14	0.15	0	0.105
X ₈								1	0.961**	-0.206	0.478**	-0.3	-0.395**	-0.025	-0.047	0.284	0.194	0.259
X ₉									1	-0.204	0.491**	-0.396**	-0.447**	-0.186	-0.089	0.243	0.089	0.216
X ₁₀										1	-0.466**	0.549**	0.577**	-0.077	-0.107	0.213	0.397**	0.301
X ₁₁											1	-0.512**	-0.525**	-0.075	-0.3	0.16	-0.02	0.172
X ₁₂												1	0.849**	-0.063	-0.061	0.163	0.370*	0.239
X ₁₃													1	-0.27	-0.004	0.192	0.355*	0.252
X ₁₄														1	0.117	-0.232	-0.114	-0.258
X ₁₅															1	0.109	0.198	0.068
X ₁₆																1	0.819**	0.944**
X ₁₇																	1	0.822**
X ₁₈																		1

X₁ : Plant height (cm)
X₂ : Number of leaves
X₃ : Scape length (cm)
X₄ : Days to flower initiation
X₅ : Number of umbels per plant
X₆ : Number of flowers per umbel
X₇ : Number of seeds per umbel
X₈ : Number of seeds per plant
X₉ : Seed yield per plant (g)
X₁₀ : Ten bulb weight (g)

X₁₁ : Total soluble solids (brix %)
X₁₂ : Equatorial diameter (cm)
X₁₃ : Polar diameter (cm)
X₁₄ : Number of centres per bulb
X₁₅ : Per cent dry weight (g)
X₁₆ : Germination (%)
X₁₇ : Seedling vigour index-I
X₁₈ : Seedling vigour index-II
 * significance @ 5 % probability level
 ** significance @ 1 % probability level

Number of umbels per plant revealed highly significant positive correlation with plant height (0.440), number of leaves (0.720), scape length (0.611), days to flower initiation (0.445), equatorial diameter (0.611), polar diameter (0.734) and seedling vigour index-I (0.463). It showed highly significant negative correlation with number of seeds per umbel (-0.432), seed yield per plant (-0.332), total soluble solids (-0.475) and significant negative correlation with seed yield per plant (-0.332)

4.5.1.2 Estimation phenotypic (p) correlation among the different seed characters.

Correlation studies were conducted to understand the relationship between various seed physical, biochemical and quality parameters. The data pertaining to phenotypic correlation coefficients for all possible pairs involving 17 characters are presented in Table 4.8 and the details are presented below:

Characters like total soluble sugars (0.408), total soluble proteins (0.502), α amylase activity (0.397), shoot length (0.437), root length (0.337), seedling length (0.442) showed highly significant positive correlation with germination (%) while bulk density (-0.104), seed length (-0.211), seed width (-0.233) showed negative correlation.

Seedling vigour index-I showed highly significant positive correlation with total soluble sugars (0.513), total soluble proteins (0.520), α amylase activity (0.465), germination (0.819), shoot length (0.801), root length (0.739), seedling length (0.876) and seedling vigour index –II (0.565) while test weight (-0.136), bulk density (-0.128), seed length (-0.088), seed width (-0.151) showed low negative correlation.

Seedling vigour index-II showed highly significant positive correlation with total soluble sugars (0.523), total soluble proteins (0.521), α amylase activity (0.479), germination (0.944), shoot length (0.468), root length (0.402), seedling length (0.496), seedling dry weight (0.565) and seedling vigour index –I (0.822) while test weight (-0.289), bulk density (-0.093), seed length (-0.226), seed width (-0.281) showed low negative correlation.

Characters like bulk density (0.422 seed length (0.656), seed width (0.645) showed highly significant positive correlation with test weight while total soluble sugars (-0.144), total soluble proteins (-0.092 germination (-0.269), seedling vigour index-II (-0.289) showed negative correlation

Total soluble sugars revealed significant positive correlation with bulk density (0.340) and highly significant positive correlation with total soluble proteins (0.474), α amylase activity (0.767), germination (0.408), shoot length (0.415), seedling length (0.455), seedling dry weight (0.510), seedling vigour index-I (0.513), seedling vigour index-II (0.523). It showed negative correlation with seed length (-0.027), seed width (-0.099).

Table 4.8: Phenotypic correlation coefficients between seed physical, biochemical and seed quality characters of onion genotypes.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇
X ₁	1	0.422**	-0.912**	0.656**	0.645**	-0.144	-0.092	-0.2	-0.196	-0.269	-0.031	0.063	0.016	-0.181	-0.136	-0.289	-0.04
X ₂		1	-0.380*	0.417**	0.394**	0.340*	0.155	0.261	-0.263	-0.104	-0.199	-0.011	-0.123	-0.021	-0.128	-0.093	0.353*
X ₃			1	-0.685**	-0.567**	0.04	0.029	0.137	0.236	0.143	-0.036	-0.105	-0.078	0.117	0.028	0.159	0.133
X ₄				1	0.805**	-0.027	-0.181	-0.128	0.014	-0.211	-0.052	0.131	0.041	-0.146	-0.088	-0.226	-0.284
X ₅					1	-0.099	-0.023	-0.18	-0.059	-0.233	-0.099	0.154	0.026	-0.25	-0.11	-0.281	-0.218
X ₆						1	0.474**	0.767**	-0.116	0.408**	0.415**	0.385*	0.455**	0.510**	0.513**	0.523**	0.289
X ₇							1	0.501**	-0.145	0.502**	0.29	0.380*	0.379*	0.262	0.520**	0.521**	0.336*
X ₈								1	-0.213	0.397**	0.299	0.373*	0.381*	0.406**	0.465**	0.479**	0.445**
X ₉									1	-0.026	0.028	-0.414**	-0.21	-0.143	-0.151	-0.072	-0.380*
X ₁₀										1	0.437**	0.337*	0.442**	0.261	0.819**	0.944**	0.255
X ₁₁											1	0.547**	0.889**	0.284	0.801**	0.468**	0.054
X ₁₂												1	0.870**	0.330*	0.739**	0.402**	0.261
X ₁₃													1	0.348*	0.876**	0.496**	0.175
X ₁₄														1	0.359*	0.565**	0.212
X ₁₅															1	0.822**	0.261
X ₁₆																1	0.292
X ₁₇																	1

X₁ : Test weight (g)X₂ : Bulk density (g/cc)X₃ : Number of seeds per gramX₄ : Seed length (mm)X₅ : Seed width (mm)X₆ : Total soluble sugars ($\mu\text{g ml}^{-1}$)X₇ : Total soluble proteins ($\mu\text{g ml}^{-1}$)X₈ : α -amylase activity ($\mu\text{g M ml/min}$)X₉ : Electrical conductivity ($\mu\text{ S ppt}^{-1}$)X₁₀ : Germination (%)X₁₁ : Shoot length (cm)X₁₂ : Root length (cm)X₁₃ : Seedling length (cm)X₁₄ : Mean seedling dry weight (mg)X₁₅ : Seedling vigour index-IX₁₆ : Seedling vigour index-IIX₁₇ : Germination after controlled deterioration (%)

* Significance @ 5 % probability level

** Significance @ 1 % probability level

Total soluble proteins revealed significant positive correlation with root length (0.380), seedling length (0.379) and highly significant positive correlation with total soluble sugars (0.474), α amylase activity (0.501), germination (0.502), seedling vigour index-I (0.520), seedling vigour index-II (0.521). It showed negative correlation with seed length (-0.181), seed width (-0.023).

α amylase activity showed highly significant positive correlation with total soluble sugars (0.767), total soluble proteins (0.501), germination (0.397), root length (0.373), seedling dry weight (0.406), seedling vigour index-I (0.465), seedling vigour index-II (0.479), and germination after controlled deterioration (0.445). It showed negative correlation with seed length (-0.128) and seed width (-0.18).

4.5.2 Path coefficient analysis

Path coefficient analysis were carried at phenotypic levels taking germination per cent, seedling vigour index I, seedling vigour index II, and seed yield per plant as dependent characters. The results pertaining to the path coefficient analyses are presented below.

Direct effect of different characters on germination per cent at phenotypic level.

At the phenotypic level, vigour index II (0.8231) recorded the highest positive direct effect on germination per cent, followed by vigour index I (0.4186) which was of considerable magnitude. Plant height (0.0104), scape length (0.0865), days to flower initiation (0.1422), number of flowers per umbel (0.1737), number of seeds per umbel (0.2760), bulk density (0.2461), alpha amylase activity (0.1323) and electrical conductivity (0.0677) recorded positive direct effects on germination per cent but their magnitude were low. However, seed yield per plant (-0.2776) recorded the highest negative direct effect on germination per cent followed by, total soluble sugars (-0.207) total soluble solids (-0.1891) number of umbels per plant (-0.1724), number of leaves (-0.1649), ten bulb weight (-0.0819), test weight (-0.044), and total soluble proteins (-0.0594) recorded negative direct effect on germination per cent but their magnitude were low (Table 4.9).

Seedling vigour index exhibited positive indirect effect on germination per cent via seedling vigour index –I (0.6806), α amylase activity (0.3964), total soluble proteins (0.4292), total soluble sugars (0.4303), ten bulb weight (0.2466) and number of umbels per plant (0.2285).

Table 4.9: Direct (diagonal) and indirect effects of plant growth, flowering, bulb and seed characters on germination % of onion genotypes.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈	X ₁₉	GER
X ₁	0.0104	0.0066	0.0044	0.0043	0.0048	0.0013	-0.002	-0.0018	0.0013	-0.0019	0.0044	0.0017	0.0028	0.0023	0.002	0.0029	-0.0047	0.0012	0.0015	0.1031
X ₂	-0.1056	-0.1649	-0.0879	-0.0508	-0.1209	0.0011	0.0692	0.0783	-0.0415	0.0538	-0.1006	-0.029	-0.0413	-0.0498	-0.0381	-0.0554	0.0889	-0.0542	-0.0366	0.1466
X ₃	0.0371	0.0461	0.0865	0.0449	0.0528	-0.013	-0.0177	-0.0093	0.0058	-0.019	0.0027	0.0226	0.0242	0.0185	0.0106	0.0038	-0.0047	0.0014	-0.0011	-0.0531
X ₄	0.0593	0.0438	0.0738	0.1422	0.0643	-0.0452	0.008	0.0445	0.0539	-0.0308	0.0202	0.0415	0.0446	0.0651	0.0675	0.0289	-0.0066	0.047	0.0418	0.263
X ₅	-0.0799	-0.1264	-0.1053	-0.078	-0.1724	0.0305	0.0747	0.0575	-0.0717	0.0819	-0.1051	-0.0457	-0.0694	-0.0902	-0.0407	-0.0692	0.0659	-0.0808	-0.0479	0.2141
X ₆	0.0215	-0.0012	-0.0261	-0.0552	-0.0307	0.1737	0.0398	0.0238	-0.0267	0.1122	-0.0132	-0.1178	-0.0565	0.0002	0.0031	0.0234	-0.0211	-0.0003	0.0272	0.143
X ₇	-0.0539	-0.1159	-0.0566	0.0155	-0.1195	0.0633	0.276	0.2529	-0.1004	0.1546	-0.109	-0.1266	-0.104	-0.049	0.0653	-0.051	0.0528	0	0.0291	0.1565
X ₈	0.0483	0.1318	0.03	-0.0868	0.0925	-0.038	-0.2544	-0.2776	0.0566	-0.1364	0.11	0.1062	0.0694	-0.0111	-0.0876	0.0177	-0.0878	-0.0249	-0.0601	0.2498
X ₉	-0.0102	-0.0206	-0.0055	-0.0311	-0.0341	0.0126	0.0298	0.0167	-0.0819	0.0382	-0.0449	-0.023	-0.0085	-0.0409	-0.023	-0.0313	0.0117	-0.0326	-0.0245	0.2099
X ₁₀	0.0339	0.0617	0.0416	0.041	0.0898	-0.1222	-0.1059	-0.0929	0.0883	-0.1891	0.0976	0.142	0.0608	0.0049	-0.0398	-0.0087	-0.0361	0.0031	-0.0327	0.1752
X ₁₁	-0.0127	-0.018	-0.0009	-0.0042	-0.018	0.0023	0.0117	0.0117	-0.0162	0.0153	-0.0296	-0.0065	-0.0069	-0.0122	-0.0037	-0.0154	0.0188	-0.0111	-0.007	0.1598
X ₁₂	-0.0072	-0.0078	-0.0115	-0.0129	-0.0117	0.0299	0.0202	0.0169	-0.0124	0.0331	-0.0097	-0.044	-0.0187	0.0064	0.004	0.009	0.0086	0.006	0.0127	-0.2656
X ₁₃	0.0674	0.0616	0.0687	0.0771	0.099	-0.08	-0.0927	-0.0615	0.0257	-0.0791	0.0573	0.1044	0.2461	0.0852	0.0412	0.0614	-0.1183	-0.031	-0.0222	-0.683
X ₁₄	-0.0458	-0.0625	-0.0444	-0.0947	-0.1083	-0.0003	0.0367	-0.0083	-0.1035	0.0053	-0.0855	0.0299	-0.0717	-0.207	-0.0987	-0.159	0.024	-0.1073	-0.1082	0.4145
X ₁₅	-0.0113	-0.0137	-0.0073	-0.0282	-0.014	-0.0011	-0.014	-0.0187	-0.0167	-0.0125	-0.0074	0.0054	-0.0099	-0.0283	-0.0594	-0.03	0.0086	-0.0309	-0.031	0.504
X ₁₆	0.0367	0.0445	0.0058	0.0269	0.053	0.0178	-0.0245	-0.0085	0.0505	0.0061	0.069	-0.0271	0.033	0.1016	0.0669	0.1323	-0.028	0.0623	0.0637	0.4169
X ₁₇	-0.0305	-0.0365	-0.0037	-0.0031	-0.0259	-0.0082	0.013	0.0214	-0.0096	0.0129	-0.0429	-0.0132	-0.0179	-0.0079	-0.0098	-0.0143	0.0677	-0.0103	-0.0049	-0.0309
X ₁₈	0.0479	0.1377	0.0068	0.1382	0.1962	-0.0007	0	0.0376	0.1667	-0.0068	0.157	-0.0566	-0.0527	0.2169	0.2176	0.1972	-0.0635	0.4186	0.3461	0.8308
X ₁₉	0.1194	0.1825	-0.0105	0.2418	0.2285	0.1289	0.0869	0.1783	0.2466	0.1424	0.1942	-0.2375	-0.0742	0.4303	0.4292	0.3964	-0.06	0.6806	0.8231	0.953

X₁ : Plant height (cm)

X₂ : Number of leaves

X₃ : Scape length (cm)

X₄ : Days to flower initiation

X₅ : Number of umbels per plant

X₆ : Number of flowers per umbel

X₇ : Number of seeds per umbel

X₈ : Seed yield per plant (g)

X₉ : Ten bulb weight (g)

X₁₀ : Total soluble solids (brix %)

X₁₁ : Equatorial diameter (cm)

X₁₂ : Test weight (g)

X₁₃ : Bulk density (g/cc)

X₁₄ : Total soluble sugars (µg ml⁻¹)

X₁₅ : Total soluble proteins (µg ml⁻¹)

X₁₆ : α-amylase activity (µg M ml/min)

X₁₇ : Electrical conductivity (µ S ppt⁻¹)

X₁₈ : Seedling vigour index-I

X₁₉ : Seedling vigour index-II

X₂₀ : Germination (%)

Direct effect of different characters on seedling vigour index –I at phenotypic level.

At the phenotypic level, germination per cent (1.0186) recorded the highest positive direct effect on seedling vigour index-I, followed by number of umbels per plant (0.4496) which was of considerable magnitude. Total soluble solids (0.2436), number of leaves (0.1533), seed yield per plant (0.1802), ten bulb weight (0.0539), equatorial diameter (0.057), test weight (0.0981), total soluble sugars (0.2296) and total soluble proteins (0.1205) recorded positive direct effects on germination per cent but their magnitude were low. However, bulk density (-0.4239) recorded the highest negative direct effect on seedling vigour index –I followed by, scape length (-0.2638) and plant height (-0.0602), days to flower initiation (-0.0414), number of flowers per umbel (-0.222), number of seeds per umbel (-0.2253), α amylase activity (-0.1059), and electrical conductivity (-0.0738) recorded negative direct effect on germination per cent but their magnitude were low (Table 4.10).

Seedling vigour index-II exhibited positive indirect effect on seedling vigour index -I via days to flower initiation (0.2678), number of umbels per plant (0.2181), seed yield per plant (0.2544), α amylase activity (0.4247), total soluble proteins (0.5134), and total soluble sugars (0.4222).

Number of umbels per plant also exhibited positive indirect effect on seedling vigour index -I via plant height (0.2314), number of leaves (0.3663), scape length (0.3052) and total soluble sugars (0.2615).

Direct effect of different characters on seedling vigour index –II at phenotypic level

At the phenotypic level, germination per cent (0.975) recorded the highest positive direct effect on seedling vigour index-II, followed by seed yield per plant (0.3858) which was of considerable magnitude. Plant height (0.0009), number of leaves (0.2148), number of umbels per plant (0.0964), ten bulb weight (0.0934), total soluble solids (0.1801), equatorial diameter (0.0291), test weight (0.0285), total soluble sugars (0.2307) and total soluble proteins (0.0864) recorded positive direct effects on germination per cent but their magnitude were low. However number of seeds per umbel (-0.352) recorded the highest negative direct effect on seedling –II, followed by days to flower initiation (-0.21) and scape length (-0.0652), number of flowers per umbel (-0.1728), bulk density (-0.2274), α amylase activity (-0.1625), electrical conductivity (-0.0682) recorded negative direct effect on germination per cent but their magnitude were low (Table 4.11).

Table: 4.10 Direct (diagonal) and indirect effects of plant growth, flowering, bulb and seed characters on seedling vigour index-I of onion genotypes

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈	X ₁₉	SVH
X ₁	-0.0602	-0.0385	-0.0258	-0.0251	-0.0279	-0.0075	0.0117	0.0105	-0.0075	0.0108	-0.0258	-0.0098	-0.0165	-0.0133	-0.0114	-0.0167	0.0272	-0.0062	-0.0087	0.1144
X ₂	0.0981	0.1533	0.0818	0.0472	0.1124	-0.001	-0.0643	-0.0728	0.0386	-0.05	0.0935	0.027	0.0384	0.0463	0.0355	0.0515	-0.0826	0.0225	0.034	0.3288
X ₃	-0.1132	-0.1407	-0.2638	-0.137	-0.1611	0.0397	0.0541	0.0285	-0.0178	0.058	-0.0081	-0.069	-0.0737	-0.0565	-0.0323	-0.0115	0.0143	0.014	0.0034	0.0164
X ₄	-0.0173	-0.0128	-0.0215	-0.0414	-0.0187	0.0131	-0.0023	-0.0129	-0.0157	0.009	-0.0059	-0.0121	-0.013	-0.0189	-0.0197	-0.0084	0.0019	-0.0109	-0.0122	0.3302
X ₅	0.2314	0.3663	0.3052	0.226	0.4996	-0.0884	-0.2163	-0.1665	0.2078	-0.2373	0.3045	0.1323	0.201	0.2615	0.1179	0.2004	-0.191	0.107	0.1387	0.4687
X ₆	-0.0275	0.0015	0.0334	0.0705	0.0393	-0.222	-0.0509	-0.0304	0.0342	-0.1434	0.0169	0.1506	0.0722	-0.0003	-0.004	-0.0299	0.0269	-0.0317	-0.0348	-0.0016
X ₇	0.044	0.0946	0.0462	-0.0127	0.0976	-0.0517	-0.2253	-0.2065	0.082	-0.1262	0.089	0.1033	0.0849	0.04	-0.0533	0.0417	-0.0431	-0.0353	-0.0238	0.0001
X ₈	-0.0313	-0.0856	-0.0195	0.0564	-0.0601	0.0247	0.1652	0.1802	-0.0368	0.0886	-0.0715	-0.069	-0.0451	0.0072	0.0569	-0.0115	0.057	0.045	0.039	0.0898
X ₉	0.0067	0.0136	0.0036	0.0204	0.0224	-0.0083	-0.0196	-0.011	0.0539	-0.0252	0.0296	0.0151	0.0056	0.0269	0.0152	0.0206	-0.0077	0.0113	0.0162	0.3983
X ₁₀	-0.0437	-0.0794	-0.0536	-0.0528	-0.1157	0.1574	0.1364	0.1197	-0.1137	0.2436	-0.1257	-0.1828	-0.0783	-0.0063	0.0513	0.0112	0.0465	0.0427	0.0421	-0.0163
X ₁₁	0.0025	0.0035	0.0002	0.0008	0.0035	-0.0004	-0.0023	-0.0023	0.0031	-0.003	0.0057	0.0013	0.0013	0.0024	0.0007	0.003	-0.0036	0.0009	0.0014	0.375
X ₁₂	0.016	0.0173	0.0257	0.0287	0.026	-0.0665	-0.045	-0.0375	0.0276	-0.0736	0.0215	0.0981	0.0416	-0.0142	-0.0089	-0.0201	-0.0191	-0.026	-0.0283	-0.1351
X ₁₃	-0.1162	-0.1061	-0.1184	-0.1329	-0.1705	0.1379	0.1597	0.106	-0.0442	0.1362	-0.0988	-0.1799	-0.4239	-0.1468	-0.071	-0.1057	0.1122	0.029	0.0382	-0.1258
X ₁₄	0.0508	0.0693	0.0492	0.105	0.1201	0.0003	-0.0407	0.0092	0.1147	-0.0059	0.0947	-0.0332	0.0795	0.2296	0.1094	0.1763	-0.0267	0.0952	0.12	0.5181
X ₁₅	0.0229	0.0279	0.0147	0.0572	0.0284	0.0022	0.0285	0.038	0.0339	0.0254	0.0151	-0.0109	0.0202	0.0574	0.1205	0.0609	-0.0175	0.0607	0.0628	0.5198
X ₁₆	-0.0294	-0.0356	-0.0046	-0.0215	-0.0425	-0.0142	0.0196	0.0068	-0.0405	-0.0049	-0.0553	0.0217	-0.0264	-0.0814	-0.0536	-0.1059	0.0225	-0.0442	-0.051	0.4712
X ₁₇	0.0333	0.0398	0.004	0.0034	0.0282	0.0089	-0.0141	-0.0233	0.0105	-0.0141	0.0468	0.0144	0.0195	0.0086	0.0107	0.0156	-0.0738	0.0023	0.0054	-0.1518
X ₁₈	0.105	0.1493	-0.0541	0.2678	0.2181	0.1457	0.1594	0.2544	0.2138	0.1784	0.1627	-0.2705	-0.0696	0.4222	0.5134	0.4247	-0.0315	1.0186	0.9707	0.8308
X ₁₉	-0.0721	-0.1102	0.0064	-0.146	-0.1379	-0.0778	-0.0524	-0.1076	-0.1489	-0.0859	-0.1172	0.1434	0.0448	-0.2598	-0.2591	-0.2393	0.0362	-0.4735	-0.4969	0.8269

X₁ : Plant height (cm)

X₂ : Number of leaves

X₃ : Scape length (cm)

X₄ : Days to flower initiation

X₅ : Number of umbels per plant

X₆ : Number of flowers per umbel

X₇ : Number of seeds per umbel

X₈ : Seed yield per plant (g)

X₉ : Ten bulb weight (g)

X₁₀ : Total soluble solids (brix %)

X₁₁ : Equatorial diameter (cm)

X₁₂ : Test weight (g)

X₁₃ : Bulk density (g/cc)

X₁₄ : Total soluble sugars (µg ml⁻¹)

X₁₅ : Total soluble proteins (µg ml⁻¹)

X₁₆ : α-amylase activity (µg M ml/min)

X₁₇ : Electrical conductivity (µ S ppt⁻¹)

X₁₈ : Germination %

X₁₉ : Seedling vigour index-II

X₂₀ : Seedling vigour index-I

Residual effect = 0.286

Table 4.11: Direct (diagonal) and indirect effects of plant growth, flowering, bulb and seed characters on seedling vigour index-II of onion genotypes.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈	X ₁₉	SVI-II
X ₁	0.0009	0.0006	0.0004	0.0004	0.0004	0.0001	-0.0002	-0.0002	0.0001	-0.0002	0.0004	0.0001	0.0002	0.0002	0.0002	0.0003	-0.0004	0.0001	0.0001	0.1451
X ₂	0.1375	0.2148	0.1146	0.0662	0.1574	-0.0015	-0.0902	-0.102	0.054	-0.0701	0.131	0.0378	0.0537	0.0649	0.0497	0.0722	-0.1158	0.0315	0.0706	0.2218
X ₃	-0.028	-0.0348	-0.0652	-0.0339	-0.0398	0.0098	0.0134	0.007	-0.0044	0.0143	-0.002	-0.0171	-0.0182	-0.014	-0.008	-0.0029	0.0035	0.0035	-0.0011	-0.0128
X ₄	-0.0876	-0.0647	-0.1091	-0.21	-0.095	0.0667	-0.0118	-0.0657	-0.0796	0.0455	-0.0298	-0.0614	-0.0658	-0.0961	-0.0997	-0.0427	0.0097	-0.0552	-0.0693	0.2938
X ₅	0.0446	0.0707	0.0589	0.0436	0.0964	-0.0171	-0.0417	-0.0321	0.0401	-0.0458	0.0587	0.0255	0.0388	0.0504	0.0228	0.0387	-0.0369	0.0206	0.0452	0.2776
X ₆	-0.0214	0.0012	0.026	0.0549	0.0306	-0.1728	-0.0396	-0.0237	0.0266	-0.1116	0.0132	0.1172	0.0562	-0.0002	-0.0031	-0.0232	0.021	-0.0247	0.0003	0.1566
X ₇	0.0687	0.1478	0.0722	-0.0198	0.1524	-0.0807	-0.352	-0.3226	0.128	-0.1972	0.139	0.1614	0.1326	0.0625	-0.0833	0.0651	-0.0674	-0.0551	0	0.1055
X ₈	-0.0671	-0.1831	-0.0416	0.1207	-0.1286	0.0528	0.3535	0.3858	-0.0787	0.1896	-0.153	-0.1476	-0.0965	0.0155	0.1218	-0.0247	0.122	0.0964	0.0347	0.2166
X ₉	0.0116	0.0235	0.0063	0.0354	0.0389	-0.0144	-0.034	-0.0191	0.0934	-0.0436	0.0512	0.0262	0.0097	0.0467	0.0263	0.0357	-0.0133	0.0196	0.0372	0.2996
X ₁₀	-0.0323	-0.0588	-0.0396	-0.039	-0.0856	0.1164	0.1009	0.0885	-0.0841	0.1801	-0.093	-0.1352	-0.0579	-0.0046	0.0379	0.0083	0.0344	0.0316	-0.0029	0.173
X ₁₁	0.0125	0.0178	0.0009	0.0041	0.0178	-0.0022	-0.0115	-0.0116	0.016	-0.015	0.0291	0.0064	0.0068	0.012	0.0036	0.0152	-0.0185	0.0047	0.0109	0.2359
X ₁₂	0.0046	0.005	0.0074	0.0083	0.0075	-0.0193	-0.013	-0.0109	0.008	-0.0214	0.0062	0.0285	0.0121	-0.0041	-0.0026	-0.0058	-0.0055	-0.0076	-0.0038	-0.2886
X ₁₃	-0.0623	-0.0569	-0.0635	-0.0713	-0.0915	0.074	0.0857	0.0569	-0.0237	0.0731	-0.053	-0.0965	-0.2274	-0.0788	-0.0381	-0.0567	0.0602	0.0155	0.0286	-0.0902
X ₁₄	0.0511	0.0697	0.0494	0.1055	0.1207	0.0003	-0.0409	0.0092	0.1153	-0.0059	0.0952	-0.0333	0.0799	0.2307	0.1099	0.1772	-0.0268	0.0956	0.1195	0.5228
X ₁₅	0.0164	0.02	0.0106	0.041	0.0204	0.0015	0.0204	0.0273	0.0243	0.0182	0.0108	-0.0078	0.0145	0.0412	0.0864	0.0437	-0.0126	0.0435	0.0449	0.5215
X ₁₆	-0.0451	-0.0546	-0.0071	-0.033	-0.0652	-0.0219	0.0301	0.0104	-0.0621	-0.0075	-0.0848	0.0333	-0.0405	-0.1248	-0.0822	-0.1625	0.0344	-0.0678	-0.0766	0.4817
X ₁₇	0.0308	0.0367	0.0037	0.0031	0.0261	0.0083	-0.013	-0.0216	0.0097	-0.013	0.0432	0.0133	0.018	0.0079	0.0099	0.0144	-0.0682	0.0021	0.0103	-0.0729
X ₁₈	0.1005	0.143	-0.0518	0.2564	0.2088	0.1394	0.1525	0.2435	0.2047	0.1708	0.1558	-0.2589	-0.0666	0.4041	0.4914	0.4065	-0.0301	0.975	0.81	0.953
X ₁₉	-0.0277	-0.0795	-0.004	-0.0799	-0.1134	0.0004	0	-0.0217	-0.0964	0.0039	-0.0907	0.0327	0.0304	-0.1253	-0.1257	-0.114	0.0367	-0.201	-0.2419	0.8269

X₁ : Plant height (cm)X₂ : Number of leavesX₃ : Scape length (cm)X₄ : Days to flower initiationX₅ : Number of umbels per plantX₆ : Number of flowers per umbelX₇ : Number of seeds per umbelX₈ : Seed yield per plant (g)X₉ : Ten bulb weight (g)X₁₀ : Total Soluble Solids (brix %)X₁₁ : Equatorial diameter (cm)X₁₂ : Test weight (g)X₁₃ : Bulk density (g/cc)X₁₄ : Total soluble sugars (µg ml⁻¹)X₁₅ : Total soluble proteins (µg ml⁻¹)X₁₆ : α-amylase activity (µg M ml/min)X₁₇ : Electrical conductivity (µ S ppt⁻¹)X₁₈ : Germination (%)X₁₉ : Seedling vigour index-IX₂₀ : Seedling vigour index-II

Residual effect = 0.2

Seedling vigour index-I exhibited positive indirect effect on seedling vigour index-II via days to flower initiation (0.22564), number of umbels per plant (0.2088), seed yield per plant (0.2435), ten bulb weight (0.2047), α amylase activity (0.4065), total soluble proteins (0.4914) and total soluble sugars (0.4041).

Number of seeds per umbel exhibited indirect positive effect on seedling vigour index-II via number of leaves (0.1478) and test weight (0.1614). Number of leaves also exhibited indirect positive effect on seedling vigour index-I via scape length (0.1146) and number of umbels per plant (0.1574). Seed yield per plant exhibited indirect positive effect via number of seeds per umbel.

Direct effect of different characters on seed yield per plant at phenotypic level.

At the phenotypic level, number of seeds per umbel (0.8226) recorded the highest positive direct effect on seed yield per plant, followed by days to flower initiation (0.2967) which was of considerable magnitude. Scape length (0.0295), number of flowers per umbel (0.1131), equatorial diameter (0.0538), test weight (0.0439), bulk density (0.0303), total soluble sugars (0.0573), α amylase activity (0.0306), electrical conductivity (0.01068), seedling vigour index-I (0.0457) and seedling vigour index-II (0.2011) recorded positive direct effects on germination per cent but their magnitude were low. However number of leaves (-0.2597), plant height (-0.0437), number of umbels per plant (-0.0028), total soluble solids (-0.0566), total soluble proteins (-0.0037) and germination percentage (-0.1714) recorded direct negative effect on seed yield per plant but their magnitude were low. (Table 4.12)

Seedling vigour index-II exhibited positive indirect effect on seed yield per plant via seedling vigour index-I (0.1663), germination per cent (0.1917), α amylase activity (0.3964), total soluble proteins (0.1049), and total soluble sugars (0.1051).

Number of umbels per plant also exhibited moderate positive indirect effect on seed yield per plant via number of flowers per umbel (0.1885), total soluble solids (0.4609), electrical conductivity (0.1574) and germination per cent (0.1287).

4.6 Heterosis

The extent of heterosis expressed in the hybrids Arka lalima and Arka kirthiman over mid parent (MP), Better parent (BP) and standard parent (SP) for different characters are presented in Table 4.13 and 4.14.

4.6.1 Arka lalima

In this hybrid maximum mid parent heterosis was exhibited by total soluble sugars (83.45) followed by α -amylase activity (74.08) and total soluble proteins (66.61). Moderate levels of mid parent heterosis was exhibited by germination per cent (13.93), seedling length (15.99), seedling vigour index-I (32.29), seedling vigour index-II (17.42)

and germination after controlled deterioration (16.41). Test weight (-6.15) exhibited low negative mid parent heterosis. While bulk density (6.58), seed width (0.24) and electrical conductivity (5.38) exhibited very low mid parent heterosis.

α -amylase activity (66.23; 56.25) exhibited highest better parent and standard heterosis respectively followed by total soluble sugars (53.31; 52.78) and total soluble proteins.

Germination per cent (12.75; 0.74), seedling length (11.50; 1.48), seedling vigour index –I (25.81; 2.18), seedling vigour index –II (14.91; 2.08) exhibited low level of better parent heterosis and standard heterosis respectively. Test weight (-11.81; -7.44) and electrical conductivity (-7.52;-2.52) and seed width (-1.42;-5.43) exhibited negative better parent and standard heterosis respectively.

4.6.2 Arka kirthiman

In this hybrid total soluble proteins (19.41) exhibited maximum mid parent heterosis followed by α -amylase activity (18.76), total soluble sugars (17.31), germination after controlled deterioration (14.38), seedling vigour index – I (13.33) and seedling vigour index –II (11.65). Germination per cent (6.82), electrical conductivity (8.41), and seedling length (6.08) exhibited low mid parent heterosis. While test weight (-16.88), bulk density (-5.26), seed length (-4.64) and seed width (-12.05) exhibited negative mid parent heterosis.

Table 4.12: Direct (diagonal) and indirect effects of plant growth, flowering, bulb and seed characters on seed yield per plant (g) of onion genotypes.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈	X ₁₉	X ₂₀
X ₁	-0.0437	-0.028	-0.0187	-0.0182	-0.0202	-0.0054	0.0085	-0.0054	0.0078	-0.0187	-0.0071	-0.012	-0.0097	-0.0083	-0.0121	0.0197	-0.0045	-0.005	-0.0063	-0.1739
X ₂	-0.1663	-0.2597	-0.1385	-0.08	-0.1904	0.0018	0.109	-0.0653	0.0847	-0.1584	-0.0457	-0.065	-0.0785	-0.0601	-0.0873	0.14	-0.0381	-0.0854	-0.0576	-0.4747
X ₃	0.0127	0.0157	0.0295	0.0153	0.018	-0.0044	-0.0061	0.002	-0.0065	0.0009	0.0077	0.0082	0.0063	0.0036	0.0013	-0.0016	-0.0016	0.0005	-0.0004	-0.1079
X ₄	0.1238	0.0915	0.1541	0.2967	0.1342	-0.0943	0.0167	0.1125	-0.0643	0.0421	0.0867	0.093	0.1358	0.1409	0.0603	-0.0137	0.078	0.098	0.0872	0.3128
X ₅	-0.0013	-0.0021	-0.0017	-0.0013	-0.0028	0.0005	0.0012	-0.0012	0.0014	-0.0017	-0.0008	-0.0011	-0.0015	-0.0007	-0.0011	0.0011	-0.0006	-0.0013	-0.0008	-0.3332
X ₆	0.014	-0.0008	-0.017	-0.0359	-0.02	0.1131	0.0259	-0.0174	0.0731	-0.0086	-0.0767	-0.0368	0.0001	0.002	0.0152	-0.0137	0.0162	-0.0002	0.0177	0.137
X ₇	-0.1605	-0.3453	-0.1687	0.0462	-0.3562	0.1885	0.8226	-0.2991	0.4607	-0.3247	-0.3772	-0.3098	-0.1459	0.1947	-0.1521	0.1574	0.1287	0.0001	0.0868	0.9164
X ₈	-0.009	-0.0182	-0.0049	-0.0274	-0.0301	0.0111	0.0263	-0.0724	0.0338	-0.0397	-0.0203	-0.0076	-0.0362	-0.0203	-0.0276	0.0103	-0.0152	-0.0288	-0.0217	-0.2041
X ₉	0.0102	0.0185	0.0125	0.0123	0.0269	-0.0366	-0.0317	0.0264	-0.0566	0.0292	0.0425	0.0182	0.0015	-0.0119	-0.0026	-0.0108	-0.0099	0.0009	-0.0098	0.4915
X ₁₀	0.0231	0.0328	0.0016	0.0076	0.0328	-0.0041	-0.0212	0.0295	-0.0278	0.0538	0.0118	0.0125	0.0222	0.0067	0.0281	-0.0341	0.0086	0.0202	0.0127	-0.3965
X ₁₁	0.0072	0.0077	0.0115	0.0128	0.0116	-0.0298	-0.0201	0.0123	-0.033	0.0096	0.0439	0.0186	-0.0063	-0.004	-0.009	-0.0086	-0.0117	-0.0059	0.0127	-0.3827
X ₁₂	0.0083	0.0076	0.0085	0.0095	0.0122	-0.0098	-0.0114	0.0032	-0.0097	0.0071	0.0128	0.0303	0.0105	0.0051	0.0076	-0.008	-0.0021	-0.0038	-0.0027	-0.2501
X ₁₃	0.0127	0.0173	0.0123	0.0262	0.03	0.0001	-0.0102	0.0287	-0.0015	0.0237	-0.0083	0.0199	0.0573	0.0273	0.044	-0.0067	0.0238	0.0297	0.03	0.0401
X ₁₄	-0.0007	-0.0009	-0.0004	-0.0017	-0.0009	-0.0001	-0.0009	-0.001	-0.0008	-0.0005	0.0003	-0.0006	-0.0018	-0.0037	-0.0019	0.0005	-0.0019	-0.0019	-0.0019	0.3158
X ₁₅	0.0085	0.0103	0.0013	0.0062	0.0123	0.0041	-0.0057	0.0117	0.0014	0.016	-0.0063	0.0076	0.0235	0.0155	0.0306	-0.0065	0.0128	0.0144	0.0147	-0.064
X ₁₆	-0.0482	-0.0576	-0.0058	-0.0049	-0.0408	-0.013	0.0204	-0.0152	0.0204	-0.0678	-0.0208	-0.0283	-0.0124	-0.0155	-0.0226	0.1068	-0.0033	-0.0162	-0.0078	0.3162
X ₁₇	-0.0177	-0.0251	0.0091	-0.0451	-0.0367	-0.0245	-0.0268	-0.036	-0.03	-0.0274	0.0455	0.0117	-0.071	-0.0864	-0.0715	0.0053	-0.1714	-0.1424	-0.1633	0.2498
X ₁₈	0.0052	0.015	0.0007	0.0151	0.0214	-0.0001	0	0.0182	-0.0007	0.0172	-0.0062	-0.0058	0.0237	0.0238	0.0216	-0.0069	0.038	0.0457	0.0378	0.0898
X ₁₉	0.0292	0.0446	-0.0026	0.0591	0.0558	0.0315	0.0212	0.0603	0.0348	0.0474	-0.058	-0.0181	0.1051	0.1049	0.0969	-0.0147	0.1917	0.1663	0.2011	0.2166

X₁ : Plant height (cm)

X₂ : Number of leaves

X₃ : Scape length (cm)

X₄ : Days to flower initiation

X₅ : Number of umbels per plant

X₆ : Number of flowers per umbel

X₇ : Number of seeds per umbel

X₈ : Ten bulb weight (g)

X₉ : Total Soluble solids (brix %)

X₁₀ : Equatorial diameter (cm)

X₁₁ : Test weight (g)

X₁₂ : Bulk density (g/cc)

X₁₃ : Total soluble sugars (µg ml⁻¹)

X₁₄ : Total soluble proteins (µg ml⁻¹)

X₁₅ : α-amylase activity (µg M ml/min)

X₁₆ : Electrical conductivity (µS ppt⁻¹)

X₁₇ : Germination %

X₁₈ : Seedling vigour index-I

X₁₉ : Seedling vigour index-II

X₂₀ : Seed yield per plant (g)

Residual effect = 0.144

Table: 4.13 Estimates of mid parent heterosis (MPH), better parent heterosis (BPH) and standard heterosis (SH) for seed characters in Arka Lalima.

PARAMETER	MPH	BPH	SH
Test weight (g)	-6.15	-11.81	-7.44
Bulk density (g/cc)	6.58	5.38	11.83
Seed length (mm)	0.00	-0.34	1.03
seed width (mm)	0.24	-1.42	-5.43
Total soluble sugars ($\mu\text{g ml}^{-1}$)	83.45	53.31	52.78
Total soluble proteins ($\mu\text{g ml}^{-1}$)	66.61	28.80	10.70
α -amylase activity ($\mu\text{g M ml/min}$)	74.08	66.23	56.45
Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	5.38	-7.52	-2.52
Germination (%)	13.93	12.75	0.74
Seedling length (cm)	15.99	11.50	1.48
Seedling vigour index-I	32.29	25.81	2.18
Seedling vigour index-II	17.42	14.91	2.08
Germination after controlled deterioration (%)	16.41	10.43	23.08

Table: 4.14 Estimates of mid parent heterosis (MPH), better parent heterosis (BPH) and standard heterosis (SH) for seed charaters in Arka Kirthiman.

PARAMETER	MPH	BPH	SH
Test weight (g)	-16.88	-20.67	3.45
Bulk density (g/cc)	-5.26	-8.91	-2.19
Seed length (mm)	-4.64	-6.49	4.35
Seed width (mm)	-12.05	-12.44	0.00
Total soluble sugars ($\mu\text{g ml}^{-1}$)	17.31	8.50	1.65
Total soluble proteins ($\mu\text{g ml}^{-1}$)	19.41	12.74	9.82
α -amylase activity ($\mu\text{g M ml/min}$)	18.76	17.40	3.62
Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	8.41	0.63	6.88
Germination (%)	6.82	6.16	1.85
Seedling length (cm)	6.08	4.24	3.64
Seedling vigour index-I	13.33	12.01	5.59
Seedling vigour index-II	11.65	11.53	4.83
Germination after controlled deterioration (%)	14.38	14.04	3.17

Highest better parent heterosis was exhibited by α -amylase activity (17.40) followed by germination after controlled deterioration (14.04), total soluble proteins (12.74), seedling vigour index-I (12.01), seedling vigour index-II (11.53), total soluble sugars (8.50) and germination per cent (6.16). Test weight (-20.67), seed width (-12.44), bulk density (-8.91) and seed length (-6.49) exhibited negative better parent heterosis. Except Bulk density (-2.19) all the other seed characters exhibited positive heterosis.

4.7 Identification of SSR molecular markers to confirm hybridity in Onion

The molecular marker approaches based on the polymerase chain reaction became the alternate methods of choice for varietal characterization, diversity studies, hybridity confirmation and seed purity testing. Among the variety of markers available, the SSR markers are preferred due to its abundance, co-dominant nature, their distribution throughout the genome and repeatability of results. In the present study onion hybrids were amplified using ten SSR primer pairs and searched for the informative markers which can differentiate the individual hybrid from its parents.

4.7.1 Confirmation of hybridity

A total of ten primers which gave clear and reproducible bands were used for confirming hybridity of the progeny. For a progeny to be a true hybrid, there must be polymorphism between the parents and polymorphic bands which are present in the parent should also be present in the hybrid.

4.7.2 SSR analysis through Agarose Gel Electrophoresis (AGE)

The DNA of both the hybrids and their parental lines used in the study were amplified using SSR (micro satellite) primer pairs and profiled in AGE. SSR length polymorphism associated with each hybrid and parental lines was assessed on 3 per cent Agarose.

Primer pairs of 10 SSR's were screened to identify a polymorphic marker to distinguish the hybrid from its parental lines. Although the PCR products of the DNA samples on the Agarose (3 %) amplified but failed to show any polymorphism among the hybrids and their parental lines. None of the primer pairs studied showed any polymorphism on the agarose gel. (Plate 6 & 7)

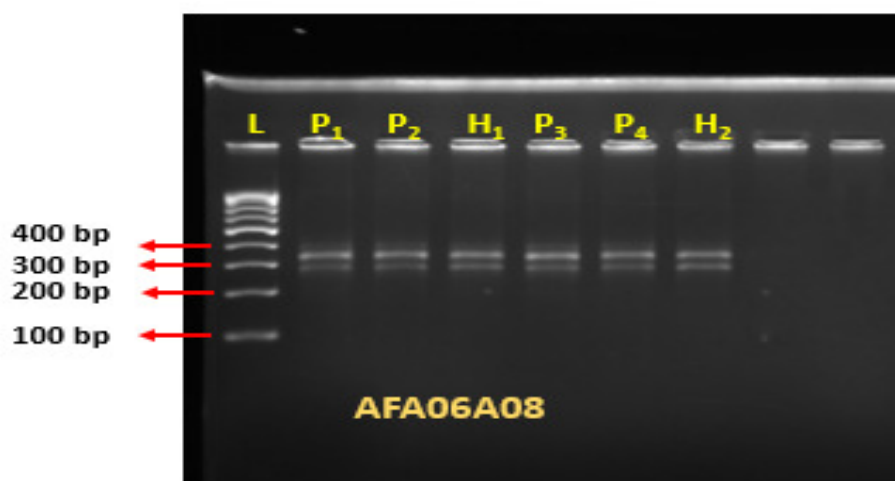
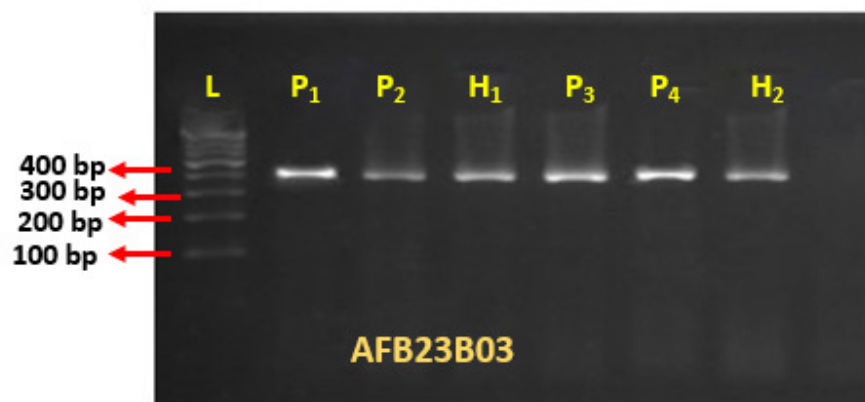


Plate 6: SSR markers AFA06A08 and AFB23B03 profile showing monomorphism on AGE (3 %)

L-ladder	
P ₁ - Female parent of Arka Lalima	P ₃ - Female parent of Arka Kirthiman
P ₂ - Male parent of Arka Lalima	P ₄ - Male parent of Arka Kirthiman
H ₁ - Hybrid-Arka Lalima	H ₂ - Hybrid-Arka Kirthiman

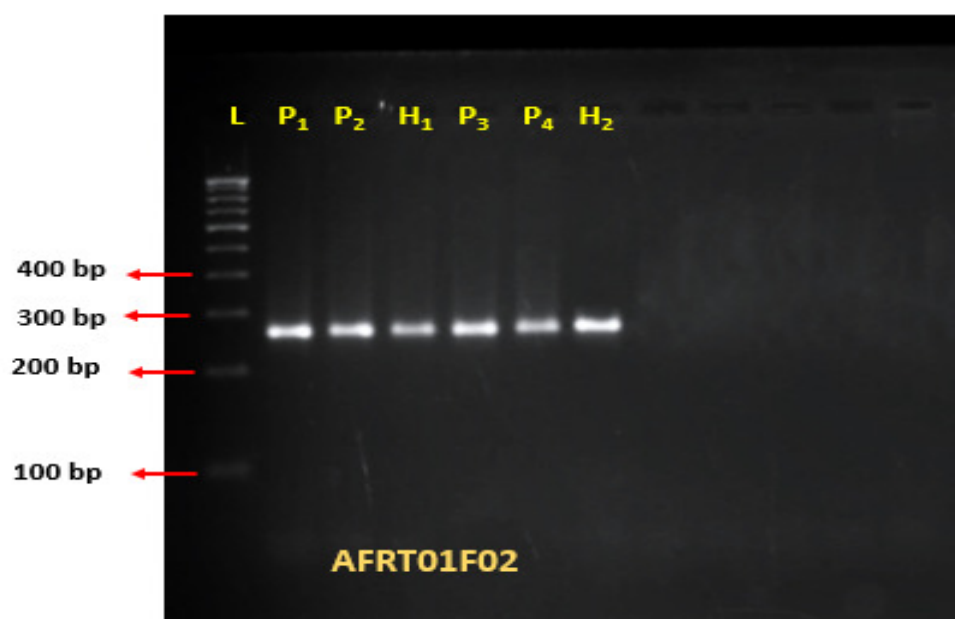
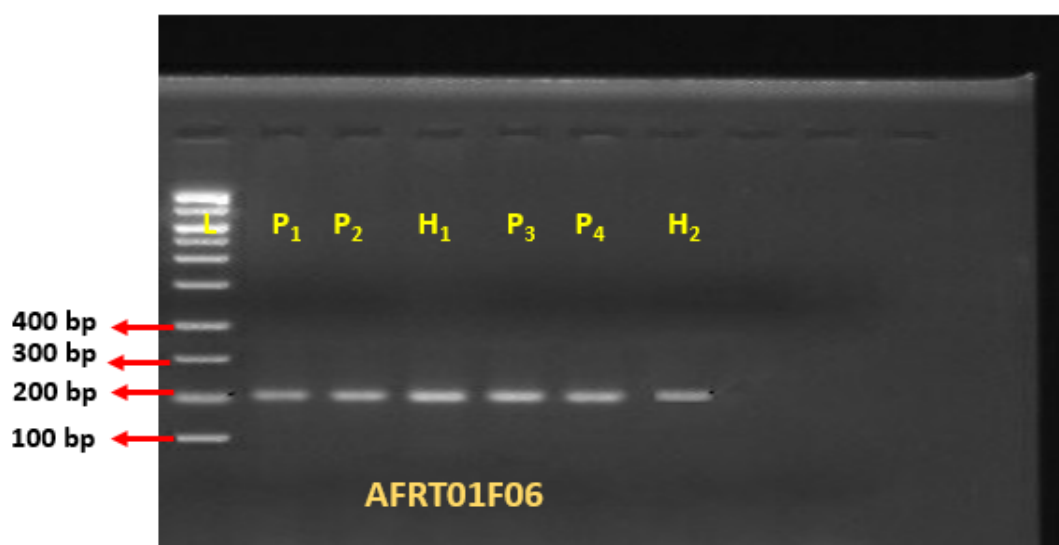


Plate 7: SSR markers AFRT01F06 and AFRT01F02 profile showing monomorphism on AGE (3 %)

L-ladder	
P ₁ - Female parent of Arka Lalima	P ₃ - Female parent of Arka Kirthiman
P ₂ - Male parent of Arka Lalima	P ₄ - Male parent of Arka Kirthiman
H ₁ - Hybrid-Arka Lalima	H ₂ - Hybrid-Arka kirthiman

V DISCUSSION

The seed quality depending on some favourable agronomic and developmental factors depends largely on the genetic factors controlling the behaviour of seed during germination and seedling growth indicating vigour.

Seedling vigour is an important attribute for the seedling establishment and is a complex character which is governed by many parameters. It is now well established that the behaviour of seed during germination and seedling vigour is polygenically controlled (Whittington, 1971; Whittington and Fierlinger, 1972). Simultaneous improvement can be made in seed quality through selection pressure as there is possibility of genetic improvement in seed quality if the breeders are supplied with the proper information on these lines during their planning of breeding programme.

In the genetic improvement of onion genotypes for seed quality and vigour, the major aspects to be considered are genetics and interrelationships of different plant growth, bulb, seed and seedling characteristics. The genetic improvement of these seed characters can be accomplished in two ways, i.e. firstly, by manipulating the genetic makeup of plants through a number of desirable, compatible genes governing the characters and secondly, by getting rid off the deleterious genes which inhibit or retard certain useful pathways leading to higher expression of these detrimental characters.

Therefore, plant breeders need basic information on the extent and nature of genetic variability available for different important seed characters and genetic association among them for the improvement of seed vigour parameters in particular crop species.

5.1. Genetic variability

5.1.1 Phenotypic Co efficient of Variation (PCV) and Genotypic Co-efficient of Variation (GCV) for different characters in onion genotypes

In the present study phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for most of the characters.

Higher value of GCV and PCV were obtained for number of seeds per plant, seed yield per plant, number of seeds per umbel, ten bulb weight, number of seeds per umbel, total soluble proteins, total soluble sugars, α amylase activity, electrical conductivity indicating the larger genotypic variability among the accessions and responsiveness of the attributes for making further improvement by selection. The smaller difference between GCV and PCV indicates that these characters were less influenced by environmental factors. These findings suggest that the above characters give a great scope for genetic improvement through selection. The estimates of PCV were higher than their corresponding GCV for all the traits that might be due to the interaction of the genotype with environment to some degree or other denoting environmental factors influencing the expression of these traits.

The low GCV and PCV was observed in plant height, scape length, per cent bulb dry weight, days to 50 per cent flowering, bulk density, seed length, seed width, seedling dry weight suggesting that less improvement can be brought about by selecting these characters. This may be due to inclusion of limited number of genotypes in the experiment which did not clearly represent all the existing variability in the characters studied. (Fig. 5.1 & 5.2)

Present results are in accordance with the earlier reports of high PCV and GCV for weight of bulb, bulb yield, number of seeds per flower by (Mohanty, 2001).

5.1.2 Heritability (broad sense) and genetic advance (%) mean

Heritability in the broad sense (bs) is the ratio of genotypic and phenotypic variance and is expressed in percentage. It gives an idea about the magnitude of environmental influence on phenotypic expression of characters. Broad sense (bs) heritability can be considered as an upper limit of the transmissibility of the characters. In the current investigation, it was quite evident that the characters showing high heritability recorded less genotypic coefficient of variability (Fig. 5.3 & 5.4).

The characters *viz.*, seed yield per plant, number of seeds per plant, number of seeds per umbel, number of seeds per flower, number of seeds per gram, total soluble proteins, total soluble sugars, α amylase activity, seedling vigour index-I had higher heritability and smaller magnitude of genotypic coefficient of variability which indicates non-additive gene action. Moderate heritability was found among characters namely plant height, scape length, seed length, seedling vigour index-II and bulk density which indicate that variability is the influence of both genetic and environmental factors. However, Borikar *et al.* (1986) in their genetic variability studies for seed vigour parameters in sorghum had reported very high heritability (bs) values for seed germination (%), shoot length, root length, seed weight, seed density, seedling vigour index and seedling dry weight.

High heritability (bs) usually permits the plant breeder to rely on phenotypic expression as an indicator to the genotypic value when the predominance of additive gene action is assumed. In early generation selection for high heritable characters can be expected to give good results. High heritability coupled with higher per cent of genetic advance is an indicator of heritability due to additive gene action (Sunil *et al.*, 2011 and Gul *et al.*, 2013).

In the present investigation, number of seeds per umbel, number of seeds per plant, number of umbels per plant, polar diameter, equatorial diameter, total soluble sugars, total soluble proteins and α amylase activity had high heritability coupled with high genetic advance which might be due to the presence of additive gene action. Hence these characters may be given ample weightage while selection process is carried on the genotypes. This is because the characters depicting high GCV are phenotypically predominant. However, these values alone are not helpful in determining the heritable portion of variation of these traits, Falconer, (1960) and Burton, (1952) suggested that the GCV together with high heritability estimates would give better picture about the extent of genetic advance to be

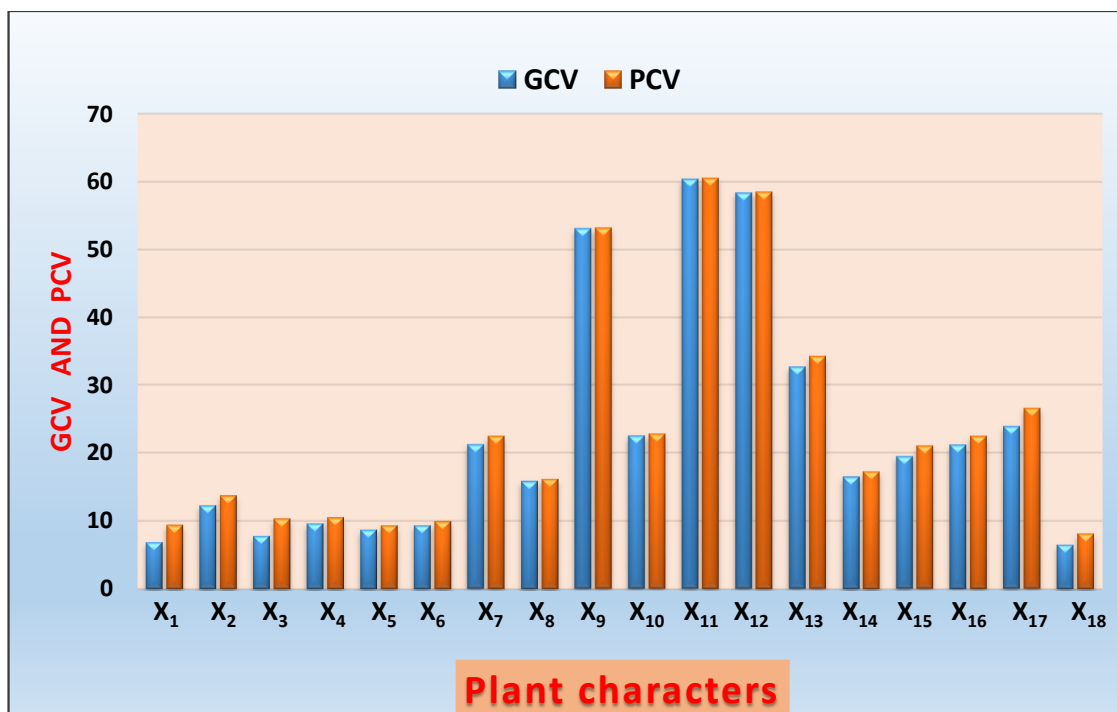


Fig. 5.1 Phenotypic and genotypic coefficients of variation of onion Plant characters.

X₁=Plant height (cm)
 X₂= Number of leaves
 X₃= Scape length (cm)
 X₄= Days to flower initiation
 X₅= Days to 50 % flowering
 X₆=Days to completion of flowering
 X₇= Number of umbels per plant
 X₈= No of flowers per umbel
 X₉= No of seeds per umbel

X₁₀= Number of seeds per flower
 X₁₁=Number of seeds per plant
 X₁₂=Seed yield per plant (g)
 X₁₃=Ten bulb weight (g)
 X₁₄=Total soluble solids (brix %)
 X₁₅=Equatorial diameter (cm)
 X₁₆=Polar diameter (cm)
 X₁₇=Number of centers per bulb
 X₁₈=Per cent dry weight (mg)

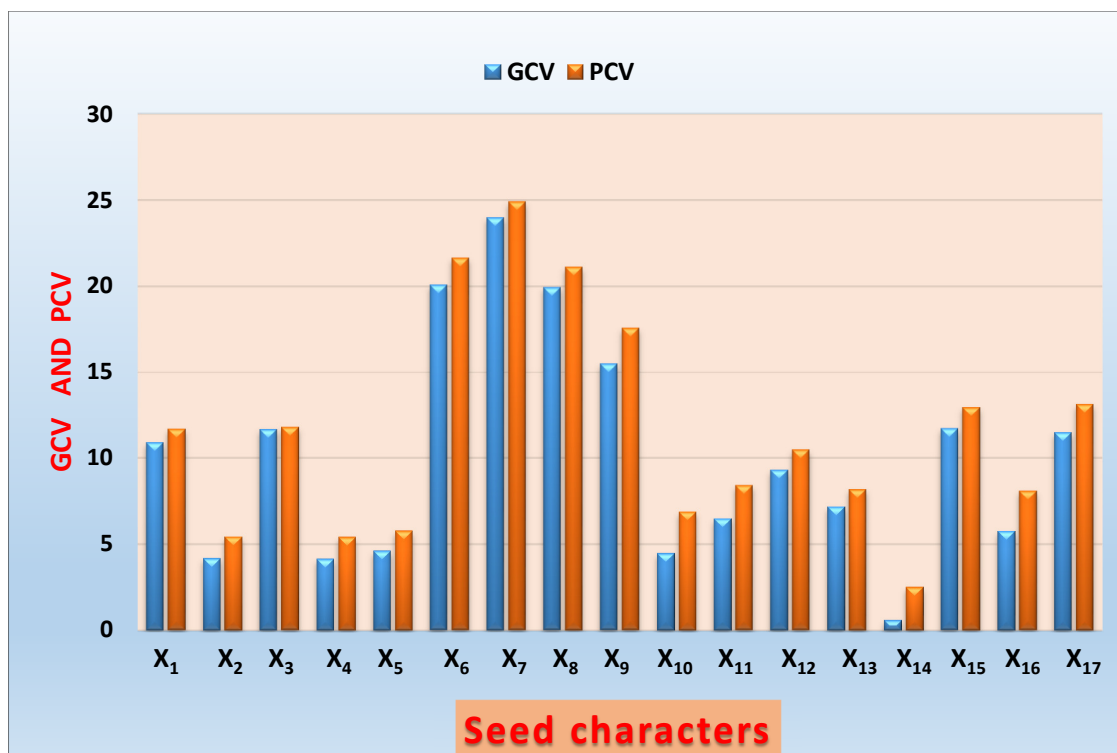


Fig. 5.2 Phenotypic and genotypic coefficients of variation of onion Seed characters.

X₁ : Test weight (g)	X₁₀ : Germination (%)
X₂ : Bulk density (g/cc)	X₁₁ : Shoot length (cm)
X₃ : Number of seeds per gram	X₁₂ : Root length (cm)
X₄ : Seed length (mm)	X₁₃ : Seedling length (cm)
X₅ : Seed width (mm)	X₁₄ : Seedling dry weight (mg)
X₆ : Total soluble sugars ($\mu\text{g ml}^{-1}$)	X₁₅ : Seedling vigour index-I
X₇ : Total soluble proteins ($\mu\text{g ml}^{-1}$)	X₁₆ : Seedling vigour index-II
X₈ : α -amylase activity ($\mu\text{g M ml/min}$)	X₁₇ : Germination after controlled deterioration (%)
X₉ : Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	

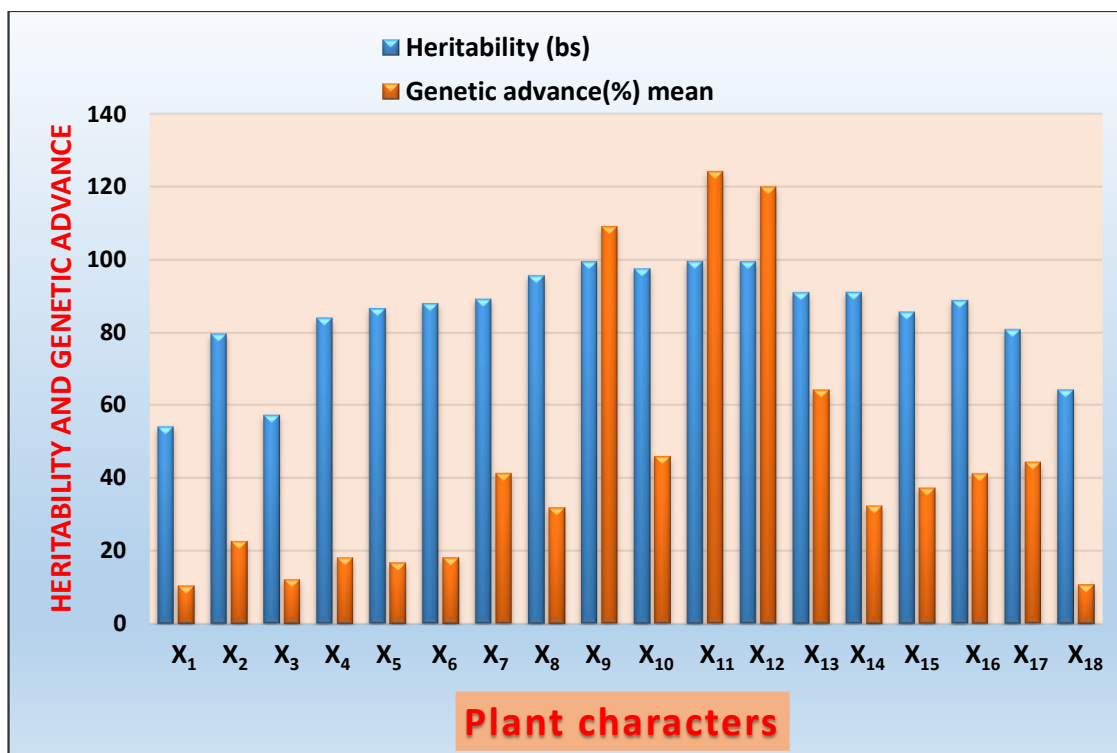


Fig. 5.3 Heritability (bs) and genetic advance (%) mean of different onion Plant characters.

X₁=Plant height (cm)

X₂= Number of leaves

X₃= Scape length (cm)

X₄= Days to flower initiation

X₅= Days to 50 % flowering

X₆=Days to completion of flowering

X₇= Number of umbels per plant

X₈= No of flowers per umbel

X₉= No of seeds per umbel

X₁₀= Number of seeds per flower

X₁₁=Number of seeds per plant

X₁₂=Seed yield per plant (g)

X₁₃=Ten bulb weight (g)

X₁₄=Total soluble solids (brix %)

X₁₅=Equatorial diameter (cm)

X₁₆=Polar diameter (cm)

X₁₇=Number of centers per bulb

X₁₈=Per cent dry weight (mg)

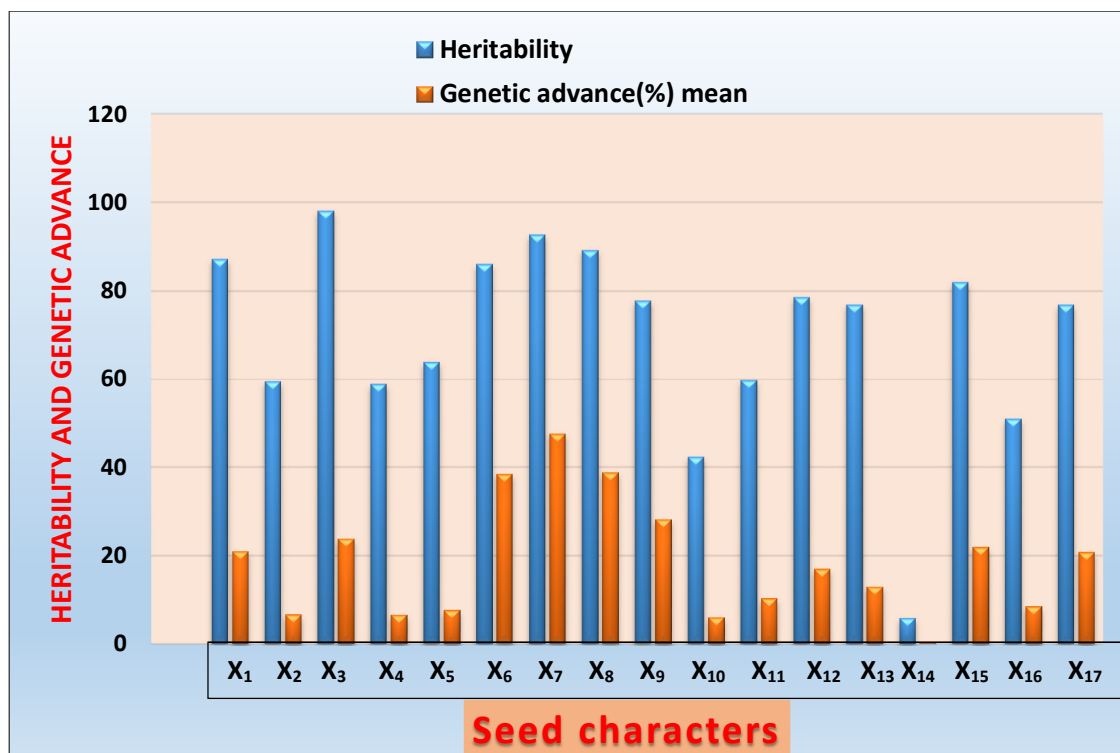


Fig. 5.4 Heritability (bs) and genetic advance (%) mean of different onion Seed characters.

X₁ : Test weight (g)	X₁₀ : Germination (%)
X₂ : Bulk density (g/cc)	X₁₁ : Shoot length (cm)
X₃ : Number of seeds per gram	X₁₂ : Root length (cm)
X₄ : Seed length (mm)	X₁₃ : Seedling length (cm)
X₅ : Seed width (mm)	X₁₄ : Seedling dry weight (mg)
X₆ : Total soluble sugars ($\mu\text{g ml}^{-1}$)	X₁₅ : Seedling vigour index-I
X₇ : Total soluble proteins ($\mu\text{g ml}^{-1}$)	X₁₆ : Seedling vigour index-II
X₈ : α -amylase activity ($\mu\text{g M ml/min}$)	X₁₇ : Germination after controlled deterioration (%)
X₉ : Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	

explored by selection. Johnson *et al.* (1955) reported that heritability in conjunction with genetic advance is more reliable in predicting the effect selection than heritable alone, because heritability estimates were subjected to genotypic and environmental interactions.

5.2 Correlation coefficient

Correlation coefficient measures the mutual relationships between characters. Information on character association among different characters is of primary importance in selection programme. Positive correlation ensures simultaneous improvement in two or more characters and negative correlation brings out the need to obtain a compromise between desirable attributes. The higher correlation between any two characters may arise due to linkage or pleiotrophism and or developmentally induced relationships. Pleiotrophy does not necessarily cause a detectable correlation because it is generated from the manifold effects of genes on more than one character (Burton, 1952).

Phenotypic correlation indicate the extent of observed relationships between the two characters while genotypic correlation provides information about linkage for the gene controlling the pair of characters (Dewey and Lu, 1959).

Therefore interpreting results of genetic association between any pair of characters, one may consider mainly the direction and magnitude of correlation values rather than their absolute values of the correlation coefficients estimates. The correlated characters are of interest for three main reasons (Falconer, 1960). Firstly in connection with the genetic cause of correlation through the pleiotrophic action of genes. Secondly, in connection with the changes brought about by selection since it is important to know the improvement in one character causing simultaneous changes in other characters. The third one is in connection with natural selection.

5.2.1 Phenotypic (p) correlation among the different plant characters.

The phenotypic data revealed that seed germination was positive and highly significantly correlated with seedling vigour index-I and seedling vigour index-II. It was positively correlated with number of leaves, days to flower initiation, number of umbels per plant, seed yield per plant, number of seeds per plant, bulb diameter and ten bulb weight. Seed germination was negatively correlated with number of centers per bulb. Similar results were quoted by Mohammad *et al.*, (2015) indicating that bulb size had significant correlation with germination percentage.

Seedling vigour index-I revealed negative correlation with number of centers per bulb and positive significant correlation with number of leaves, days to flower initiation and equatorial bulb diameter. It showed highly significant positive correlation with number of umbels per plant. The characters such as number of leaves, days to flower initiation, number of umbels per plant, number of seeds per plant, ten bulb weight and polar bulb diameter showed positive correlation with seedling vigour index-II. It

showed negative correlation with number of centers per bulb. Similar results were quoted by Sharnkumar and Basvegowda, (2014). (Fig. 5.5)

Polar bulb diameter showed highly significant positive correlation with plant height, number of leaves, number of umbels per plant, ten bulb weight, equatorial diameter and significant positive correlation with scape length. It revealed significant negative correlation with number of seeds per umbel, number of flowers per umbel, seed yield per plant and total soluble solids. Similar results were reported by Aliyu *et al.*, (2007) where positively correlated with number of leaves (0.657) and bulb weight (0.645).

Characters like number of flowers per umbel, number of seeds per umbel, number of seeds per plant, and seed yield per plant showed highly significant positive correlation with total soluble solids in bulbs. It showed significant negative correlation with number of leaves and highly significant negative correlation with number of umbels per plant, equatorial diameter, polar diameter and ten bulb weight. Per cent dry weight showed negative correlation with total soluble solids and ten bulb weight, similar results were given by Ashok *et al.*, (2013) where bulb dry weight is negatively correlated with total soluble solids (-0.221) and weight of bulb (-0.859)

Ten bulb weight showed highly significant positive correlation with number of umbels per plant, equatorial bulb diameter, polar bulb diameter, seedling vigour index-I and highly significant negative correlation with total soluble solids. It showed negative correlation with seed yield per plant. However experimental results of Kaiser *et al.*, 2003 showed that single bulb weight had highly significant positive correlation with yield per plant (0.8661) and positive correlation with diameter of bulb (0.7217)

Seed yield per plant revealed highly significant positive correlation with number of seeds per umbel, number of seeds per plant, and total soluble solids. It showed significant positive correlation with days to flower initiation and highly significant negative correlation with number of leaves, equatorial diameter and polar diameter. Also low negative correlation was observed with plant height however experimental results of Latha *et al.*, (2014) showed that seed yield per plant had positive correlation in chick pea (0.3352).

Characters like plant height, number of leaves, days to flower initiation, number of umbels per plant showed highly significant positive correlation with scape length while number of flowers per umbel, number of seeds per umbel, number of seeds per plant, number of centers per bulb showed negative correlation.

Number of umbels per plant revealed highly significant positive correlation with plant height, number of leaves, scape length, days to flower initiation, equatorial diameter, polar diameter and seedling vigour index-I. It showed highly significant negative correlation with number of seeds per umbel, seed yield per plant, total soluble solids and significant negative correlation with seed yield per plant. However it was found that plant

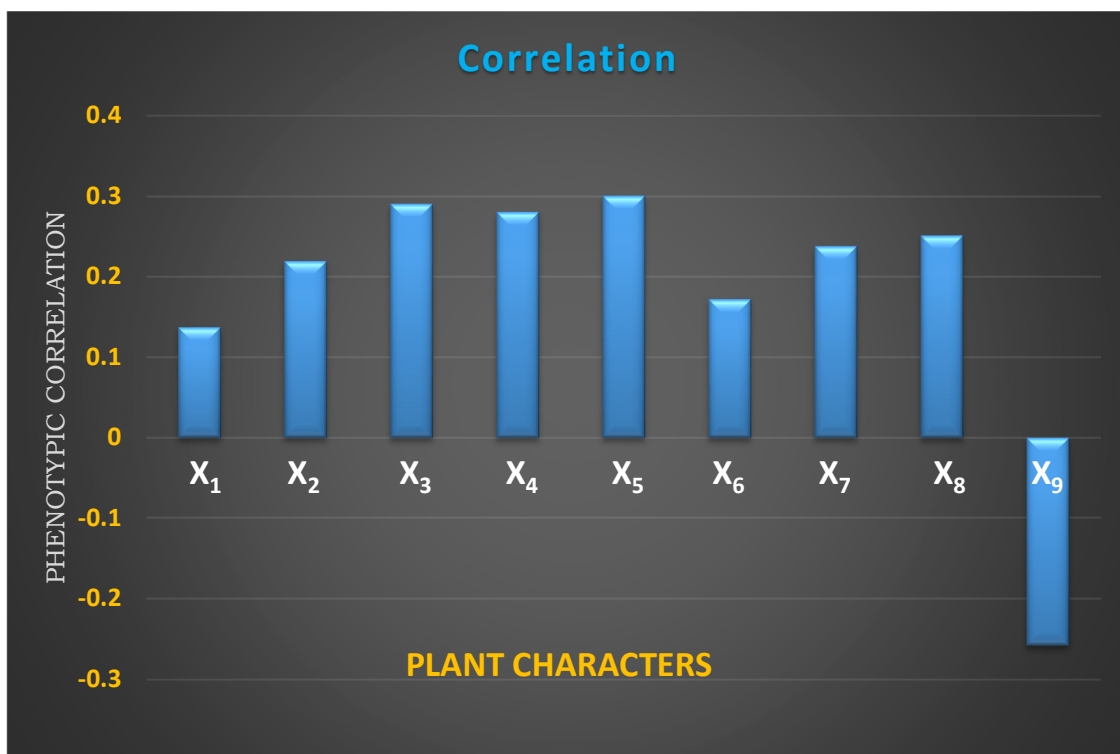


Fig. 5.5 Correlation coefficients of plant characters with Seedling vigour index-II

X₁= Plant height (cm)
 X₂= Number of leaves
 X₃= Days to flower initiation
 X₄= Days to 50 % flowering
 X₅= Ten bulb weight (g)
 X₆= Number of seeds per plant
 X₇= Seed yield per plant (g)
 X₈= Total soluble solids (brix %)
 X₉= Number of centers per bulb

height showed negative correlation with number of umbels in carrot as reported by Singh and Prasad, (2006).

5.2.3 Phenotypic (p) correlation among different seed characters.

Correlation studies were conducted to understand the relationship between various seed physical, biochemical and quality parameters. The data pertaining to phenotypic correlation coefficients for all possible pairs involving 17 characters are presented in Table 4.3 and the details are presented below:

Characters like total soluble sugars, total soluble proteins, α amylase activity, shoot length, root length (0.337), seedling length (0.442), test weight showed highly significant positive correlation with germination (%) while bulk density (-0.104), seed length (-0.211), seed width (-0.233) showed negative correlation. Positive correlation of germination percentage with test weight (0.901) was also revealed by Gamiley *et al.*, (1990). Similar results were reported by Latha C.N., (2014) where germination percentage was positively correlated with protein content (0.365) and negatively correlated with seed length (-0.325), breadth (-0.716) and width (-0.711).

Seedling vigour index-I showed highly significant positive correlation with total soluble sugars (0.513), total soluble proteins (0.520), α amylase activity (0.465), germination (0.819), shoot length (0.801), root length (0.739), seedling length (0.876) and seedling vigour index -II (0.565) while test weight (-0.136), bulk density (-0.128), seed length (-0.088), seed width (-0.151), electrical conductivity showed low negative correlation.

Seedling vigour index-II showed highly significant positive correlation with total soluble sugars (0.523), total soluble proteins (0.521), α amylase activity (0.479), germination (0.944), shoot length (0.468), root length (0.402), seedling length (0.496), seedling dry weight (0.565) and seedling vigour index -I (0.822) while test weight (-0.289), bulk density (-0.093), seed length (-0.226), seed width (-0.281) showed low negative correlation. Ravi rathod (2011) reported similar results in sunflower, where in vigour indices showed negative correlation with bulk density, electrical conductivity. (Fig. 5.6)

Characters like bulk density (0.422 seed length (0.656), seed width (0.645) showed highly significant positive correlation with test weight while total soluble sugars (-0.144), total soluble proteins (-0.092 germination (-0.269), seedling vigour index-II (-0.289) showed negative correlation.

Total soluble sugars revealed significant positive correlation with bulk density (0.340) and highly significant positive correlation with total soluble proteins (0.474), α amylase activity (0.767), germination (0.408), shoot length (0.415), seedling length (0.455), seedling dry weight (0.510), seedling vigour index-I (0.513), seedling vigour index-II (0.523). It showed negative correlation with seed length (-0.027), seed width (-0.099).

Total soluble proteins revealed significant positive correlation with root length (0.380), seedling length (0.379) and highly significant positive correlation with total soluble sugars (0.474), α amylase activity (0.501), germination (0.502), seedling vigour index-I (0.520), seedling vigour index-II (0.521). It showed negative correlation with seed length (-0.181), seed width (-0.023).

α amylase activity showed highly significant positive correlation with total soluble sugars (0.767), total soluble proteins (0.501), germination (0.397), root length (0.373), seedling dry weight (0.406), seedling vigour index-I (0.465), seedling vigour index-II (0.479), and germination after controlled deterioration (0.445). It showed negative correlation with seed length (-0.128) and seed width (-0.18).

5.3 Path coefficient analysis

Direct and indirect effect of different characters on germination per cent at phenotypic level

At the phenotypic level, vigour index I and vigour index -II recorded the highest positive direct effect on germination per cent. Plant height, scape length, days to flower initiation, number of flowers per umbel, number of seeds per umbel, bulk density, α amylase activity and electrical conductivity recorded positive direct effects on germination per cent but their magnitude were low. However, seed yield per plant recorded the highest negative direct effect on germination per cent followed by, total soluble sugars, total soluble solids, number of umbels per plant, number of leaves, ten bulb weight, test weight, and total soluble proteins recorded negative direct effect on germination per cent but their magnitude were low. (Fig. 5.7)

The negative direct effects of α amylase activity, total soluble sugars, total soluble proteins, ten bulb weight, and number of umbels per plant will be nullified by their indirect positive effects which are of high magnitude. Hence these characters can also be considered as contributing factors to germination per cent.

The lower residual effect indicated the cause and effect relationship between germination and physical, chemical characters attributes included in the analysis. Among the plant characters number of seeds per umbel directly and positively contributes to the germination percentage. Majority of the bulb characters are contribute negatively to the germination.

Degewione *et al.*, (2011) reported similar results in shallot mentioning that sprouting per cent had direct positive effect of plant height (0.2143) and direct negative effect of total soluble solids (-0.1346).

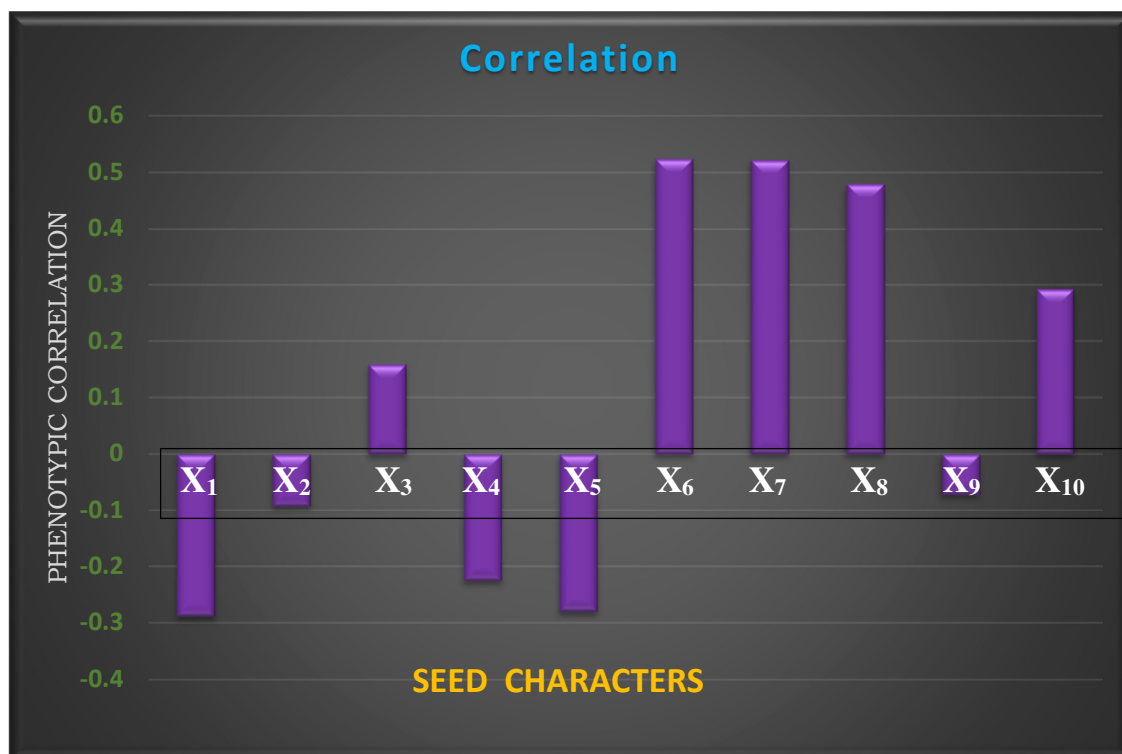


Fig. 5.6 Correlation coefficients of seed characters with Seedling vigour index-II

- X₁** : Test weight (g)
X₂ : Bulk density (g/cc)
X₃ : Number of seeds per gram
X₄ : Seed length (mm)
X₅ : Seed width (mm)
X₆ : Total soluble sugars ($\mu\text{g ml}^{-1}$)
X₇ : Total soluble proteins ($\mu\text{g ml}^{-1}$)
X₈ : α -amylase activity ($\mu\text{g M ml/min}$)
X₉ : Electrical conductivity ($\mu\text{ S ppt}^{-1}$)
X₁₀ : Germination after controlled deterioration (%)

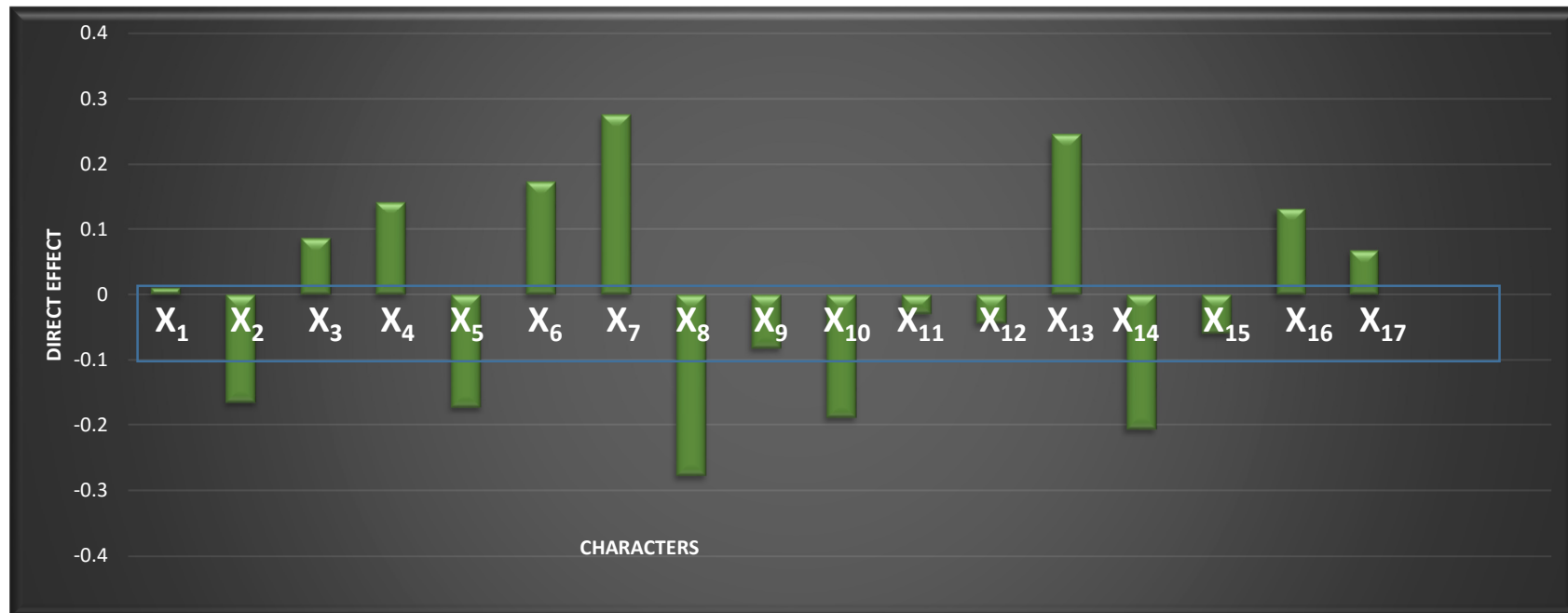


Fig. 5.7 Direct effect of plant and seed characters on germination per cent of onion genotypes.

X ₁	: Plant height (cm)	X ₁₁	: Equatorial diameter (cm)
X ₂	: Number of leaves	X ₁₂	: Test weight (g)
X ₃	: Scape length (cm)	X ₁₃	: Bulk density (g/cc)
X ₄	: Days to flower initiation	X ₁₄	: Total soluble sugars ($\mu\text{g ml}^{-1}$)
X ₅	: Number of umbels per plant	X ₁₅	: Total soluble proteins ($\mu\text{g ml}^{-1}$)
X ₆	: Number of flowers per umbel	X ₁₆	: α -amylase activity ($\mu\text{g M ml/min}$)
X ₇	: Number of seeds per umbel	X ₁₇	: Electrical conductivity ($\mu\text{ S ppt}^{-1}$)
X ₈	: Seed yield per plant (g)		
X ₉	: Ten bulb weight (g)		
X ₁₀	: Total soluble solids (brix %)		

Direct and indirect effect of different characters on seedling vigour index –I at phenotypic level

At the phenotypic level, germination per cent and number of umbels per plant recorded the highest positive direct effect on seedling vigour index-I. Total soluble solids, number of leaves, seed yield per plant, ten bulb weight, equatorial diameter, test weight, total soluble sugars and total soluble proteins recorded positive direct effects on germination per cent but their magnitude were low. Bulk density recorded the highest negative direct effect on seedling vigour index –I followed by, scape length and plant height, days to flower initiation, number of flowers per umbel, number of seeds per umbel, amylase activity, and electrical conductivity at low magnitudes. (Fig. 5.8)

These low negative direct effects will have no impact on seedling vigour index-I as their indirect positive effects are of high magnitude which would nullify their negative contribution

Direct and indirect effect of different characters on seedling vigour index –II at phenotypic level

At the phenotypic level, germination per cent and seed yield per plant recorded the highest positive direct effect on seedling vigour index-II, Plant height, number of leaves, number of umbels per plant, ten bulb weight, total soluble solids, equatorial diameter, test weight, total soluble sugars and total soluble proteins recorded positive direct effects on germination per cent but their magnitudes were low. However number of seeds per umbel recorded the highest negative direct effect on seedling –II, followed by days to flower initiation and scape length, number of flowers per umbel, bulk density, α amylase activity, electrical conductivity recorded negative direct effect on germination per cent but their magnitudes were low. (Fig. 5.9)

Latha C.N., 2014 mentioned that seed size (0.9103), seed length (0.7711) had highest direct effect on vigour index. Protein content (0.2411), α amylase activity (0.3245), seed density (0.2051) had low positive direct effect on vigour index.

Although number of seeds per umbel (-0.352) had direct negative effect it was found moderately contributing to seedling vigour index-II via number of leaves (0.148) and test weight (0.1478) which would minimize its effect. Days to flower initiation, α amylase activity and scape length exhibited indirect positive effects at high magnitudes which would nullify their direct negative effects.

Direct and indirect effect of different characters on seed yield per plant at phenotypic level.

At the phenotypic level, number of seeds per umbel recorded the highest positive direct effect on seed yield per plant, followed by days to flower initiation which was of considerable magnitude. Scape length, number of flowers per umbel, equatorial diameter,

test weight, bulk density, total soluble sugars, α amylase activity, electrical conductivity, seedling vigour index-I and seedling vigour index-II recorded positive direct effects on germination per cent but their magnitude were low. However number of leaves, plant height, number of umbels per plant, total soluble solids, total soluble proteins and germination per cent recorded direct negative effect on seed yield per plant but their magnitude were low. (5.10)

Direct negative effect of number of umbels per plant, total soluble proteins, total soluble solids and germination per cent on seed yield per plant would get masked by their indirect positive effects at high magnitudes. Similar results were quoted by Kaiser *et al.*, (2003) in onion where in it was mentioned that the genotypic correlation between yield and diameter of bulb was positive (0.6023) mainly due to the positive direct effect of single bulb weight (0.1326), dry matter contents (0.0489) and negative indirect effect of number of leaves per plant (-0.1903).

Findings of Akter *et al.*, (2012) also suggested that number of seeds per umbel (0.26), number of flowers per umbel (1.52) and test weight (0.743) exhibited direct positive effect on seed yield per umbel having high correlation with number of seeds per umbel (0.98) in onion.

5.4 Seed quality attributes linked to heterosis.

After estimating the extent of heterosis expressed in the hybrids over mid parent (MP), better parent (BP) and standard parent (SP) for different seed characters. It was observed that α -amylase activity, total soluble proteins, total soluble sugars and germination after controlled deterioration exhibited maximum mid parent heterosis (MPH), better parent heterosis (BPH), and standard heterosis (SH). Seedling length and seedling vigour indices also exhibited moderate heterosis levels. On the other hand test weight and bulk density exhibited negative heterosis. (Fig. 5.11 & 5.12)

5.5 Use of SSR markers to distinguish hybrids and its parental lines.

Characterization and identification of cultivars are crucial to varietal improvement, release and in seed production programme. It is mandatory to maintain the genetic purity of hybrid seed for the successful crop production. Unambiguous of characteristic pattern of hybrids can be obtained using DNA markers and had been termed as DNA finger printing. The use of DNA markers to obtain genotype specific profiles had distinct advantages over morphological and biochemical methods. The morphological markers are influenced by the environmental conditions, labour intensive and time consuming. However, the biochemical markers such as isozyme and protein patterns are least influenced by the environment but exhibit limited polymorphism and often do not allow discrimination between closely related inbred lines (Cooke, 1984 and Lucchese *et al.*, 1999). DNA markers overcome most of the disadvantages of morphological and biochemical markers and can be useful to distinguish varieties and offtypes. The



Fig. 5.8 Direct effect of plant and seed characters on seedling vigour index-I of onion genotypes.

X ₁	: Plant height (cm)	X ₁₁	: Equatorial diameter (cm)
X ₂	: Number of leaves	X ₁₂	: Test weight (g)
X ₃	: Scape length (cm)	X ₁₃	: Bulk density (g/cc)
X ₄	: Days to flower initiation	X ₁₄	: Total soluble sugars ($\mu\text{g ml}^{-1}$)
X ₅	: Number of umbels per plant	X ₁₅	: Total soluble proteins ($\mu\text{g ml}^{-1}$)
X ₆	: Number of flowers per umbel	X ₁₆	: α -amylase activity ($\mu\text{g M ml/min}$)
X ₇	: Number of seeds per umbel	X ₁₇	: Electrical conductivity ($\mu\text{ S ppt}^{-1}$)
X ₈	: Seed yield per plant (g)		
X ₉	: Ten bulb weight (g)		
X ₁₀	: Total soluble solids (brix %)		

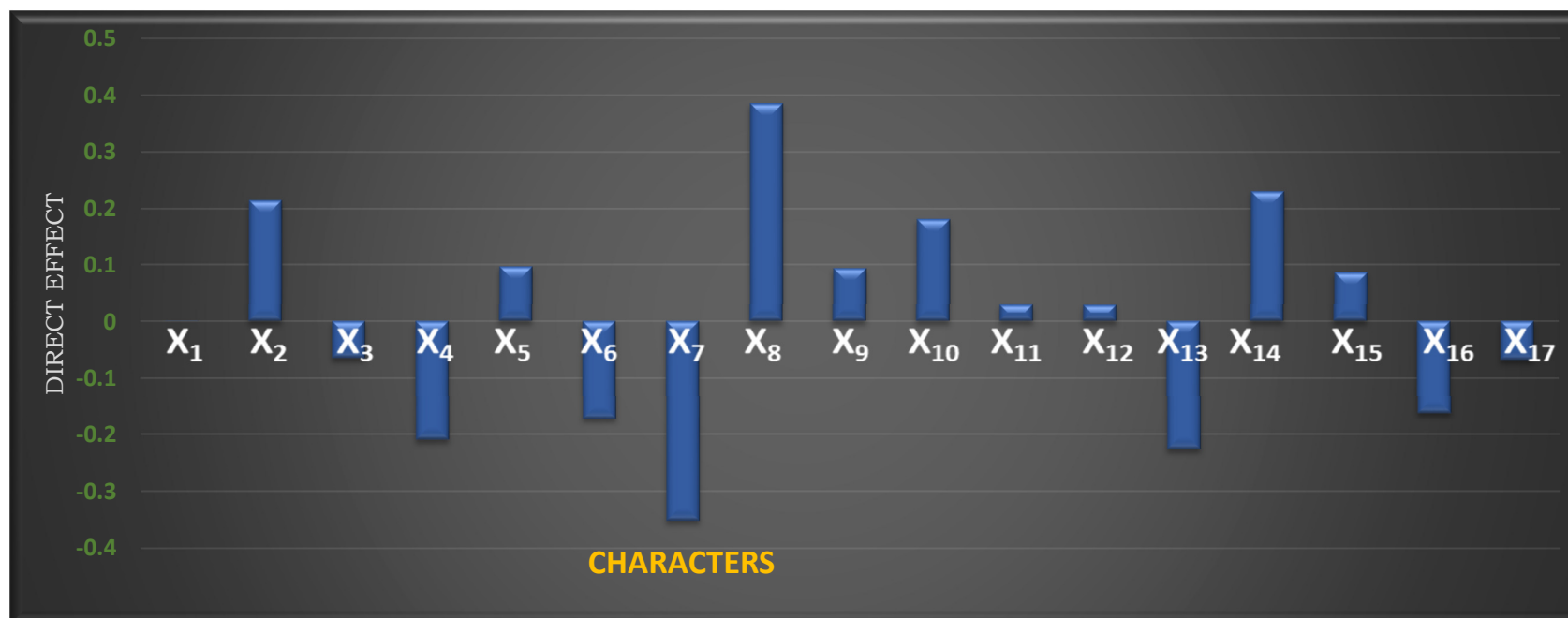


Fig. 5.9 Direct effect of plant and seed characters on seedling vigour index-II of onion genotypes.

X ₁	: Plant height (cm)	X ₁₁	: Equatorial diameter (cm)
X ₂	: Number of leaves	X ₁₂	: Test weight (g)
X ₃	: Scape length (cm)	X ₁₃	: Bulk density (g/cc)
X ₄	: Days to flower initiation	X ₁₄	: Total soluble sugars (µg ml ⁻¹)
X ₅	: Number of umbels per plant	X ₁₅	: Total soluble proteins (µg ml ⁻¹)
X ₆	: Number of flowers per umbel	X ₁₆	: α-amylase activity (µg M ml/min)
X ₇	: Number of seeds per umbel	X ₁₇	: Electrical conductivity (µ S ppt ⁻¹)
X ₈	: Seed yield per plant (g)		
X ₉	: Ten bulb weight (g)		
X ₁₀	: Total soluble solids (brix %)		

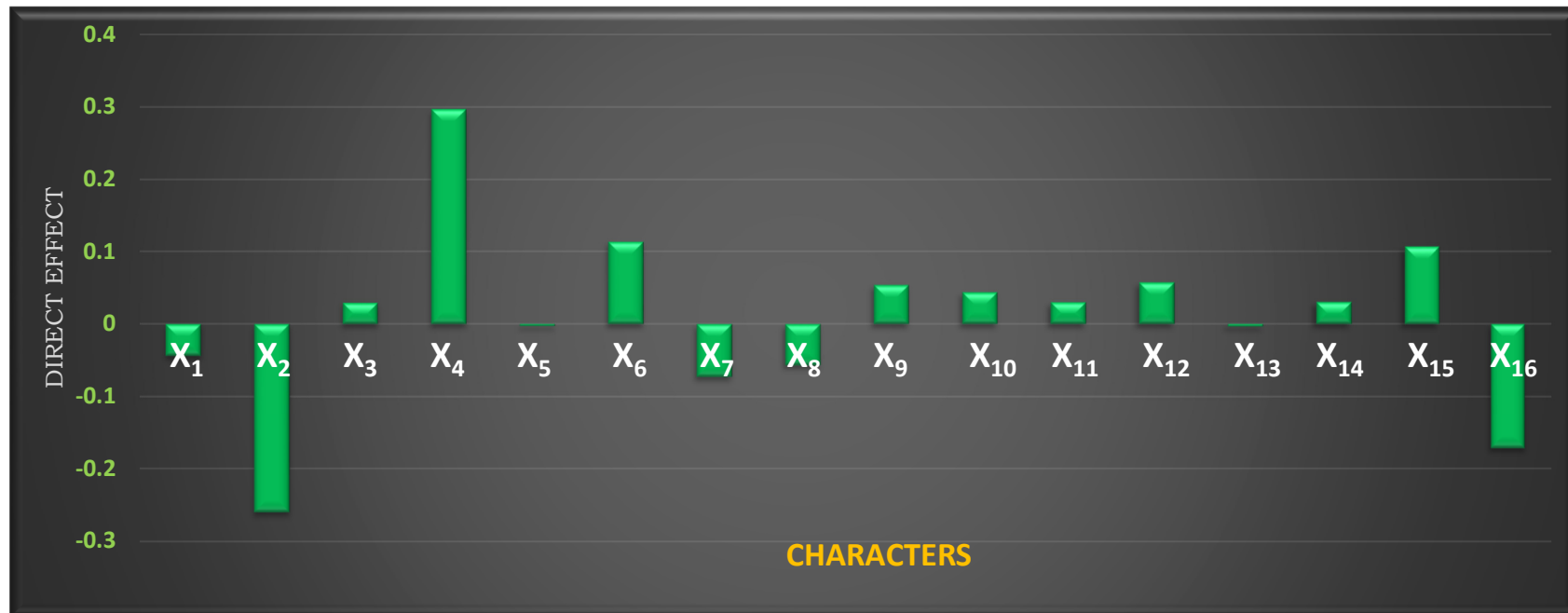


Fig. 5.10 Direct effect of plant and seed characters on seed yield per plant of onion genotypes.

X₁ : Plant height (cm)
 X₂ : Number of leaves
 X₃ : Scape length (cm)
 X₄ : Days to flower initiation
 X₅ : Number of umbels per plant
 X₆ : Number of flowers per umbel
 X₇ : Seed yield per plant (g)
 X₈ : Ten bulb weight (g)
 X₉ : Total soluble solids (brix %)
 X₁₀ : Equatorial diameter (cm)

X₁₁ : Test weight (g)
 X₁₂ : Bulk density (g/cc)
 X₁₃ : Total soluble sugars ($\mu\text{g ml}^{-1}$)
 X₁₄ : Total soluble proteins ($\mu\text{g ml}^{-1}$)
 X₁₅ : α -amylase activity ($\mu\text{g M ml/min}$)
 X₁₆ : Electrical conductivity ($\mu\text{ S ppt}^{-1}$)

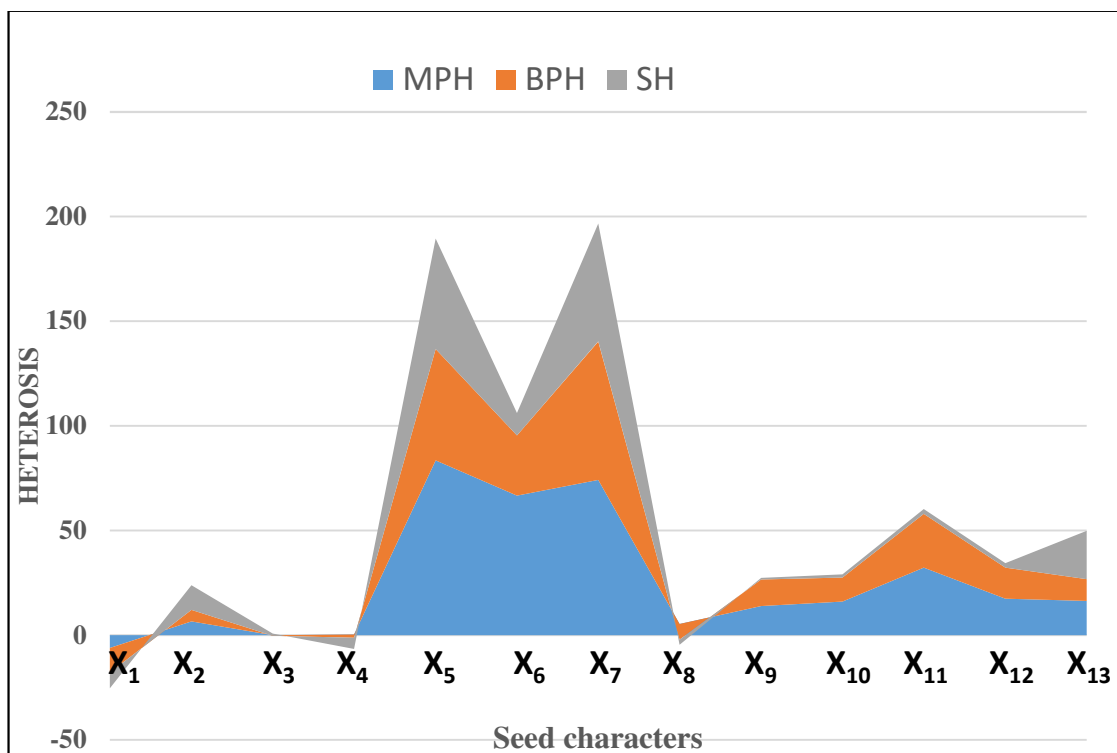


Fig. 5.11 Mid parent (MPH), better parent (BPH) and standard parent (SH) heterosis levels of seed characters in Arka Lalima.

X₁	Test weight (g)	X₈	Electrical conductivity (μ S ppt ⁻¹)
X₂	Bulk density (g/cc)	X₉	Germination (%)
X₃	Seed length (mm)	X₁₀	Seedling length (cm)
X₄	seed width (mm)	X₁₁	Seedling vigour index-I
X₅	Total soluble sugars (μ g ml ⁻¹)	X₁₂	Seedling vigour index-II
X₆	Total soluble proteins (μ g ml ⁻¹)	X₁₃	Germination after controlled deterioration (%)
X₇	α -amylase activity (μ g M ml/min)		

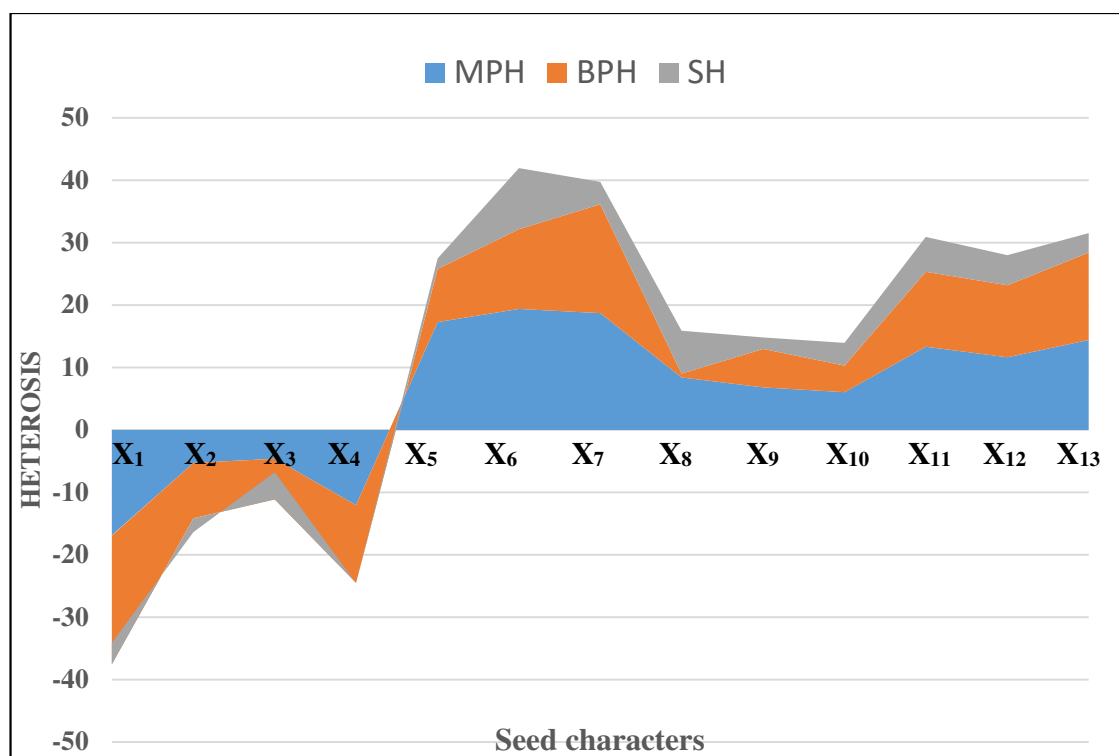


Fig. 5.12 Mid parent (MPH), better parent (BPH) and standard parent (SH) heterosis levels of seed characters in Arka Kirthiman.

X₁	Test weight (g)	X₈	Electrical conductivity (μ S ppt ⁻¹)
X₂	Bulk density (g/cc)	X₉	Germination (%)
X₃	Seed length (mm)	X₁₀	Seedling length (cm)
X₄	seed width (mm)	X₁₁	Seedling vigour index-I
X₅	Total soluble sugars (μ g ml ⁻¹)	X₁₂	Seedling vigour index-II
X₆	Total soluble proteins (μ g ml ⁻¹)	X₁₃	Germination after controlled deterioration (%)
X₇	α -amylase activity (μ g M ml/min)		

usefulness of DNA fingerprinting technique for cultivar identification was demonstrated by Dallas (1988) for the first time in rice.

The present study utilized the SSR marker techniques for identification of two hybrids along with their parental lines, demonstrating that this technique can be successfully applied to distinguish and identify the hybrids from its parental lines. SSR had much more polymorphism than most of other DNA markers, and is co-dominant and larger in quantity. Therefore, the high polymorphic information content of SSR had prompted the application of microsatellites as molecular markers in fingerprinting (Ashikawa *et al.*, 1999).

In this study 10 SSR primer pairs associated with each hybrid and parental lines were assessed on 3 per cent agarose. Although the PCR products of the DNA samples on the agarose did appeared but failed to show any polymorphism among the hybrids and its parental lines. Probably the differences in the base pair sizes are too small to resolve and identify on the agarose.

However, these primers may be expected to further and clearly amplify on polyacrylamide gel electrophoresis (PAGE) 6 per cent. Polymorphism can also be expected in few of the primers used in this study. Similar results were reported by Pallavi, (2009) wherein, 56 primer pairs used failed to amplify on 3 per cent agarose gel electrophoresis (AGE) but successfully amplified and showed polymorphism in 6 per cent polyacrylamide gel electrophoresis (PAGE).

VI SUMMARY

The present investigation was carried out at Division of Vegetable Crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89; Department of Seed Science & Technology and Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore, involving 14 genotypes of onion. The experiment was laid out in RCBD with three replication during Rabi season 2015. The main objective of this study was to estimate the components of variability (genotypic and phenotypic), heritability, correlation coefficient, path coefficient, heterosis levels among different characters and selection of characters for exercising selection pressure for good germination vigour levels in onion genotypes. The results of the study are summarized below.

Higher value of GCV and PCV were obtained for number of seeds per plant (60.44, 60.54), seed yield per plant (58.40, 58.51), number of seeds per umbel (53.13, 53.25), ten bulb weight (32.63, 34.19), total soluble proteins (23.99, 24.92), total soluble sugars (20.09, 21.65), α amylase activity (19.95, 21.12), electrical conductivity (15.52, 17.59) indicating the larger genotypic variability among the accessions and responsiveness of the attributes for making further improvement by selection.

The characters *viz.*, seed yield per plant (99.6), number of seeds per plant (99.7), number of seeds per umbel (99.6), number of seeds per flower (97.6), number of seeds per gram (98.10), total soluble proteins (92.7), total soluble sugars (86.1), α amylase activity (89.2), seedling vigour index-I (82.0) had higher heritability and smaller magnitude of genotypic coefficient of variability which indicates non-additive gene action. Moderate heritability was found among characters namely plant height, scape length, seed length, seedling vigour index-II and bulk density which indicate that variability is the influence of both genetic and environmental factors.

Number of seeds per umbel (99.6; 109.22), number of seeds per plant (99.7; 124.30), number of umbels per plant (89.3; 41.3), polar diameter (89.0; 41.24), equatorial diameter (85.8; 37.23), total soluble sugars (86.1; 38.41), total soluble proteins (92.7; 47.57) and α amylase activity (89.2; 38.81) had high heritability coupled with high genetic advance indicating the presence of additive gene action. Hence these characters may be given ample weightage while selection process is carried on the genotypes. This is because the characters depicting high GCV are phenotypically predominant.

Seed germination was positive and highly significantly correlated with seedling vigour index-I (0.819) and seedling vigour index-II (0.944). It was positively correlated with number of leaves (0.135), days to flower initiation (0.254), number of umbels per plant (0.228), seed yield per plant (0.243), number of seeds per plant (0.284), bulb diameter (0.163) and ten bulb weight (0.213). Seed characters like total soluble sugars, total soluble proteins, α amylase activity, shoot length, root length, seedling length, test weight also showed highly significant positive correlation with germination (%)

Seedling vigour index-I showed highly significant positive correlation with total soluble sugars (0.513), total soluble proteins (0.520), α amylase activity (0.465),

germination per cent (0.819), shoot length (0.801), root length (0.739), seedling length (0.876) and seedling vigour index –II while test weight (-0.136), bulk density (-0.128), seed length (-0.088), seed width (-0.11), electrical conductivity showed low negative correlation. Seedling vigour index-I revealed negative correlation with plant characters like number of centers per bulb (-0.114) and positive significant correlation with number of leaves (0.328), days to flower initiation (0.330) and equatorial bulb diameter (0.370). It showed highly significant positive correlation with number of umbels per plant (0.463). The characters such as number of leaves, days to flower initiation, number of umbels per plant, number of seeds per plant, ten bulb weight and polar bulb diameter showed positive correlation with seedling vigour index-II. It showed negative correlation with number of centers per bulb.

Seedling vigour index-II showed highly significant positive correlation with total soluble sugars (0.523), total soluble proteins (0.521), α amylase activity (0.479), germination (0.944), shoot length (0.468), root length (0.402), seedling length (0.496), seedling dry weight (0.565) and seedling vigour index –I (0.822) while test weight (-0.289), bulk density -0.093), seed length (-0.226) and seed width (-0.281) showed low negative correlation.

Path-coefficient analysis revealed the complex inter-relationships among the different characters. When seed germination was considered for path analysis plant height, scape length days to flower initiation, number of flowers per umbel, number of seeds per umbel, bulk density, α - amylase activity recorded direct positive effects.

When seedling vigour indices were considered for path analysis germination per cent and number of umbels per plant recorded highest positive direct effect. While total soluble sugars, total soluble proteins, total soluble solids, ten bulb weight, number of leaves recorded low positive direct effect.

When seed yield per plant was considered for path analysis number of seeds per umbel recorded the highest positive direct, while scape length, number of flowers per umbel, equatorial diameter, test weight, total soluble sugars, total soluble solids, α - amylase activity recorded low positive direct effect.

However characters like seed yield per plant, bulk density, number seeds per umbel posed negative direct effect on most of the seed quality attributes.

Similar results were obtained after estimating heterosis for different seed characters. Total soluble sugars, total soluble proteins and α amylase activity showed maximum heterosis over better parent, mid parent and standard parent. Characters like test weight and bulk density showed low negative heterosis.

The results so obtained here may form a basis on which onion breeder can rely upon making selections for improvement of seedling vigour while yield improvement are under way.

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Appendix-I Characters studied to assess the variability and association among different onion genotypes.

Genotypes	Plant height (cm)	Number of leaves	Scape length (cm)	Days to flower initiation	Days to flower initiation	Days to completion of flowering
G₁ : Female parent of Arka Lalima	64.7	12.1	55.1	38.1	56.2	71.6
G₂ : Male parent of Arka Lalima	59.5	11.2	58.7	39.3	62.7	75.0
G₃ : Maintainer line of Arka Lalima	63.0	11.9	55.5	39.0	57.4	69.9
G₄ : Arka Lalima F ₁ hybrid	66.0	12.5	62.0	46.9	71.3	74.6
G₅ : Female parent of Arka Kirthiman	64.1	12.4	62.7	47.3	65.4	74.5
G₆ : Male parent of Arka Kirthiman	64.4	12.0	66.3	42.5	66.1	76.3
G₇ : Maintainer line of Arka Kirthiman	64.0	11.4	67.5	46.5	62.4	76.0
G₈ : Arka Kirthiman F ₁ hybrid	65.0	13.5	62.5	47.4	67.0	77.5
G₉ : Arka Kalyan (OPV)	67.8	12.1	60.8	51.3	71.0	82.3
G₁₀ : Arka Nikethan (OPV)	63.0	11.7	63.1	46.0	65.9	85.9
G₁₁ : Prem (OPV)	54.0	9.4	61.5	46.3	62.3	68.4
G₁₂ : Arka Bindu (OPV)	66.8	10.4	60.5	43.6	65.3	72.3
G₁₃ : Arka Pragathi (OPV)	55.4	8.0	48.1	42.8	69.5	86.2
G₁₄ : Arka Ujwal (OPV)	52.5	9.8	53.4	36.1	51.3	58.4
Mean	62.15	11.32	59.8	43.8	63.8	74.9
C.V. (%)	6.37	6.20	6.76	4.19	3.42	3.44
S.Em±	2.29	0.41	2.34	1.06	1.26	1.49
C.D. (p=0.05)	6.65	1.18	6.80	3.08	3.67	4.33

Appendix-I Characters studied to assess the variability and association among different onion genotypes.

Genotypes	Number of umbels per plant	Number of flowers per umbel	Number of seeds per umbel	No. of seeds per flower	No. of seeds per plant	Seed yield per plant (g)
G₁ : Female parent of Arka Lalima	2.87	283.1	381.8	3.88	835.7	2.34
G₂ : Male parent of Arka Lalima	3.56	307	270.9	3.64	759.5	2.26
G₃ : Maintainer line of Arka Lalima	3.55	267	215	2.93	642	2.15
G₄ : Arka Lalima F ₁ hybrid	4.55	290	180	2.20	1123	3.74
G₅ : Female parent of Arka Kirthiman	3.87	196.6	221.7	2.54	800.6	2.37
G₆ : Male parent of Arka Kirthiman	4.08	334.4	300.9	3.25	971.9	3.66
G₇ : Maintainer line of Arka Kirthiman	3.95	220	187	2.53	763	3.19
G₈ : Arka Kirthiman F ₁ hybrid	4.24	310	200	2.82	946	3.01
G₉ : Arka Kalyan (OPV)	3.72	276	688	4.37	2595	9.15
G₁₀ : Arka Nikethan (OPV)	4.15	279.2	835.8	4.52	3755.3	10.70
G₁₁ : Prem (OPV)	2.78	215	520	3.94	2234	8.08
G₁₂ : Arka Bindu (OPV)	2.52	351	608	2.40	1919	8.08
G₁₃ : Arka Pragathi (OPV)	2.22	298	544	3.20	2319	8.59
G₁₄ : Arka Ujwal (OPV)	2.48	316	625	3.42	2216	6.19
Mean	3.47	281.69	412.7	3.26	1562.87	5.25
C.V. (%)	7.36	3.38	3.56	3.49	3.53	3.60
S.Em±	0.15	5.49	8.47	0.07	31.86	0.11
C.D. (p=0.05)	0.43	15.97	24.63	0.19	92.61	0.32

Appendix-I Characters studied to assess the variability and association among different onion genotypes.

Genotypes	Ten bulb weight (g)	Total soluble solids (brix %)	Equatorial diameter (cm)	Polar diameter (cm)	Number of centers per bulb	Percent dry weight (g)
G₁ : Female parent of Arka Lalima	296.67	10.52	5.71	4.54	5.27	8.01
G₂ : Male parent of Arka Lalima	433.33	10.54	5.53	4.84	3.93	8.31
G₃ : Maintainer line of Arka Lalima	253.33	10.07	5.31	4.63	3.40	9.47
G₄ : Arka Lalima F ₁ hybrid	438.33	11.57	6.24	5.90	2.80	9.15
G₅ : Female parent of Arka Kirthiman	305.00	10.36	4.86	4.58	4.07	9.63
G₆ : Male parent of Arka Kirthiman	286.67	11.21	4.42	4.25	4.13	8.33
G₇ : Maintainer line of Arka Kirthiman	266.67	9.30	4.77	4.40	3.97	9.44
G₈ : Arka Kirthiman F ₁ hybrid	453.33	12.20	5.47	4.84	3.77	7.81
G₉ : Arka Kalyan (OPV)	420.00	11.20	5.28	4.63	3.17	8.99
G₁₀ : Arka Nikethan (OPV)	240.00	12.49	5.36	4.47	4.00	8.60
G₁₁ : Prem (OPV)	346.67	10.70	3.20	2.84	4.17	9.05
G₁₂ : Arka Bindu (OPV)	118.33	15.80	3.38	3.21	1.73	7.90
G₁₃ : Arka Pragathi (OPV)	373.33	12.90	4.44	3.60	3.73	8.46
G₁₄ : Arka Ujwal (OPV)	156.67	15.83	3.22	2.40	5.67	9.33
Mean	313.45	11.76	4.80	4.22	3.84	8.75
C.V. (%)	10.20	5.13	7.93	7.45	11.61	4.88
S.Em±	18.46	0.35	0.22	0.18	0.26	0.25
C.D. (p=0.05)	53.65	1.01	0.64	0.53	0.75	0.72

Appendix-I Characters studied to assess the variability and association among different onion genotypes.

Genotypes	Test weight (g)	Bulk density (g/cc)	No. of seeds per gram	Seed length (mm)	Seed width (mm)	Total soluble sugars ($\mu\text{g ml}^{-1}$)
G₁ : Female parent of Arka Lalima	3.81	511.33	268.00	2.95	2.12	161.96
G₂ : Male parent of Arka Lalima	3.35	499.76	306.33	2.93	2.05	241.25
G₃ : Maintainer line of Arka Lalima	3.43	489.50	288.00	2.91	2.01	204.28
G₄ : Arka Lalima F ₁ hybrid	3.37	538.83	291.67	2.94	2.10	369.84
G₅ : Female parent of Arka Kirthiman	4.16	549.05	241.67	2.97	2.24	238.39
G₆ : Male parent of Arka Kirthiman	3.79	506.83	272.00	3.09	2.25	202.60
G₇ : Maintainer line of Arka Kirthiman	4.16	521.25	243.67	3.15	2.26	207.00
G₈ : Arka Kirthiman F ₁ hybrid	3.31	500.15	302.67	2.89	1.97	258.67
G₉ : Arka Kalyan (OPV)	3.63	481.82	271.00	2.92	2.22	242.09
G₁₀ : Arka Nikethan (OPV)	3.19	511.36	305.33	2.76	1.98	254.46
G₁₁ : Prem (OPV)	3.65	500.51	273.33	2.97	2.09	218.97
G₁₂ : Arka Bindu (OPV)	2.94	519.03	347.33	2.62	1.94	211.73
G₁₃ : Arka Pragathi (OPV)	3.53	495.47	260.00	3.06	2.09	222.72
G₁₄ : Arka Ujwal (OPV)	2.86	453.77	353.67	2.81	2.12	197.08
Mean	3.51	505.62	287.48	2.93	2.10	230.79
C.V. (%)	4.19	3.47	1.63	3.48	3.48	8.08
S.Em$\pm$	0.08	10.13	2.71	0.06	0.04	10.76
C.D. (p=0.05)	0.25	29.45	7.88	0.17	0.12	31.29

Appendix-I Characters studied to assess the variability and association among different onion genotypes.

Genotypes	Total soluble proteins ($\mu\text{g ml}^{-1}$)	α-amylase activity ($\mu\text{g M ml/min}$)	Electrical conductivity ($\mu\text{ S ppt}^{-1}$)	Germination (%)	Shoot length (cm)	Root length (cm)
G₁ : Female parent of Arka Lalima	501.28	140.69	109.41	79.33	7.88	6.88
G₂ : Male parent of Arka Lalima	273.76	128.48	144.90	81.00	9.07	6.94
G₃ : Maintainer line of Arka Lalima	300.22	147.27	142.50	88.67	9.97	7.09
G₄ : Arka Lalima F ₁ hybrid	645.66	233.87	134.07	91.33	10.23	7.61
G₅ : Female parent of Arka Kirthiman	528.36	127.26	117.67	85.67	9.81	7.42
G₆ : Male parent of Arka Kirthiman	469.34	124.35	137.40	86.67	9.60	7.03
G₇ : Maintainer line of Arka Kirthiman	295.36	114.21	157.00	76.33	9.16	6.24
G₈ : Arka Kirthiman F ₁ hybrid	595.69	149.41	138.27	92.00	10.29	7.67
G₉ : Arka Kalyan (OPV)	583.25	149.50	137.47	90.67	10.01	7.57
G₁₀ : Arka Nikethan (OPV)	542.43	144.18	129.37	90.33	9.93	7.41
G₁₁ : Prem (OPV)	527.82	126.12	206.67	85.33	9.49	6.09
G₁₂ : Arka Bindu (OPV)	450.45	139.32	156.77	83.67	8.41	5.33
G₁₃ : Arka Pragathi (OPV)	436.50	128.85	145.33	86.67	9.49	6.54
G₁₄ : Arka Ujwal (OPV)	469.34	125.77	168.42	85.67	9.72	7.18
Mean	472.82	141.38	144.66	85.95	9.51	6.93
C.V. (%)	6.75	6.94	8.29	5.25	5.35	4.85
S.Em $\pm$	18.42	5.66	6.92	2.60	0.29	0.19
C.D. (p=0.05)	53.54	16.46	20.12	7.57	0.85	0.56

Appendix -I Characters studied to assess the variability and association among different onion genotypes

Genotypes	Seedling length (cm)	Mean Seedling dry weight (mg)	Seedling vigour index-I	Seedling vigour index-II	Germination after controlled deterioration (%)
G₁ : Female parent of Arka Lalima	14.76	1.14	1168	90	61.6
G₂ : Male parent of Arka Lalima	16.01	1.17	1295	94	55.3
G₃ : Maintainer line of Arka Lalima	17.06	1.17	1510	103	54.6
G₄ : Arka Lalima F ₁ hybrid	17.84	1.19	1629	108	63.1
G₅ : Female parent of Arka Kirthiman	17.24	1.16	1475	99	57.0
G₆ : Male parent of Arka Kirthiman	16.63	1.15	1441	99	56.6
G₇ : Maintainer line of Arka Kirthiman	15.40	1.15	1171	87	54
G₈ : Arka Kirthiman F ₁ hybrid	17.97	1.21	1652	111	65
G₉ : Arka Kalyan (OPV)	17.59	1.17	1594	106	55.3
G₁₀ : Arka Nikethan (OPV)	17.34	1.18	1565	106	63
G₁₁ : Prem (OPV)	15.58	1.15	1330	98	50
G₁₂ : Arka Bindu (OPV)	13.74	1.16	1149	96	60
G₁₃ : Arka Pragathi (OPV)	16.02	1.17	1388	101	40
G₁₄ : Arka Ujwal (OPV)	16.90	1.16	1448	99	48
Mean	16.43	1.17	1415	100	55.9
C.V. (%)	3.94	2.45	5.49	5.68	6.30
S.Em±	0.37	0.02	44.86	3.28	2.04
C.D. (p=0.05)	1.09	-	130.41	9.55	5.92

APPENDIX- II

**Meterological data recorded for the period of research at ICAR- Indian Institute of Horticultural Research,
Hessaraghatta, Bengaluru- 89 (2015-16)**

Month	Temperature (°C)		Relative Humidity (%)		Mean wind speed (km/h)	Total Rainfall (mm)
	Max.	Min.	7.30hrs	14.00hrs		
June 2015	30.5	21.4	78.2	53.4	8.5	15.0
July 2015	30.4	21	77.7	50.6	5.8	36.0
August 2015	30.0	20.10	79.2	49.5	4.3	84.0
September 2015	30.1	19.8	80	50.8	3.4	145.2
October 2015	30.7	20.60	76.1	52.3	3.3	142.3
November 2015	28.5	18.5	84.2	52.2	3.19	43.0
December 2015	28.8	17.5	78.3	51.5	2.5	2.0
January 2016	27.2	15.10	73.3	44.8	3.2	-
February 2016	30.50	16.40	87.00	40.00	3.3	2.20
March 2016	32.70	19.20	87.00	37.00	3.3	-
April 2016	34.60	21.80	85.00	35.00	3.4	22.00
May 2016	33.20	21.10	87.00	44.00	4.2	52.80
June 2016	28.10	19.70	90.00	50.00	7.6	82.4.