# HARVESTING, POST HARVEST HANDLING AND CONDITIONING OF FRUIT AND SEED IN ASH GOURD

(Benincasa hispida (Thunb.) Cogn)

Thesis submitted in part fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) IN SEED SCIENCE AND TECHNOLOGY to the Tamil Nadu Agricultural University, Coimbatore – 641 003

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# DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY TAMIL NADU AGRICULTURAL UNIVERSITY COIMBATORE – 641 003

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**CERTIFICATE** 

This is to certify that the thesis entitled "HARVESTING, POST HARVEST

HANDLING AND CONDITIONING OF FRUIT AND SEED IN ASH GOURD

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for the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) IN SEED SCIENCE

AND TECHNOLOGY to the Tamil Nadu Agricultural University, Coimbatore, is a

record of bonafide research work carried out by Mr. P. MURUGESAN under my

supervision and guidance and that no part of this thesis has been submitted for the

award of any other degree, diploma, fellowship or other similar titles or prizes and that

the work has not been published in part or full in any scientific or popular journal or

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## Acknowledgement

It gives me immense pleasure to place on record my deep sense of gratitude and indebtedness to **Dr. K. Vanangamudi**, Professor and Head, Department of Seed Science and Technology, and Chairman of the Advisory Committee for his inspiring guidance, constant encouragement and help shown in the course of this investigation and meticulous care taken in the preparation of the thesis.

Grateful thanks are due to **Dr. D. Veeraragavathatham**, Director, Planning and monitoring, **Dr. N. Natarajan**, Professor, Department of Seed Science and Technology and **Dr. R. Balasaraswathi**, Professor and Head, Department of Plant Biochemistry, members of the advisory committee for their valuable suggestions and pains taking efforts to go through the manuscript.

I am thankful to **Th. P. Natesan**, Assistant Professor and **Dr. M. Bhaskaran**, Associate Professor and PG Coordinator, for their ungrudging co-operation and encouragement in the progress of this work.

I express my profound gratitude to **Dr. A. Bharathi**, and **Dr. R. Umarani**, **Dr. R. Jerlin**, **Dr. S. Sundereswaran**, Assistant Professors, Department of Seed Science and Technology for the kindness and encouragement they had bestowed upon me.

I express my personal thanks to **Dr. Mallika Vanangamudi** for the suggestions and encouragement rendered during the preparation of thesis material.

I express my sincere acknowledgement to authorities in Sugarcane Breeding

Institute and Central Institute of Cotton Research, Coimbatore for providing facility
to conduct experiment on microtome and electrophoresis studies.

I acknowledge the personal help from **Dr. B. Sundara**, Head, Crop Production division, **Dr. P.N. Gururaja Rao**, Principal Scientist (Crop Physiology), **Dr. C. Sankaranarayanan**, Scientist (Nematology), **R. Kamalakannan**, Research

Associate, A. Flora, R. Shanmugavadivu, Ph.D. Scholars from Sugarcane Breeding Institute.

I also acknowledge the help of **Dr. K. Ratnavel**, Senior Scientist (Seed Technology), **Dr. K. Raja**, Research Associate, **Dr. R. Anderson** from Central Institute of Cotton Research, Coimbatore.

I express my sincere thanks to **all the staff members** of Department of Seed Science and Technology for the help rendered during my study period.

I place my special thanks to **Dr. P. Rethinam**, Former Director, presently Executive Director, Asia Pacific Coconut Community, Jakartha and **Dr. M. Kochu**Babu, Director National Research Centre for Oil Palm (ICAR), Pedavegi, West Godavari district of Andhra Pradesh for the permission and grant of study leave to pursue higher study.

I thank Mr. K.R. Ranagaraj, Kondamuthunur, Sathyamangalam taluk of Erode district for providing necessary help during On Farm Trial.

I express my heartfelt thanks to all my classmates and juniors for their significant contribution during the course of this study.

I am much thankful to our Post Graduates, Ph.D. Scholars, Renugadevi, G. Shasthri, Kathiravana, student friends S. Kavitha, C. Vanitha, K. Nelson and R. Vijayan, S. Sunganthi for checking manuscript.

I am thankful to Ms. S. Sasikala, M/S Mother Computers for flawless typing and neat execution of this thesis.

I am indebted to my **Parents** who through their prayers have been a reservoir of strength and support throughout my life. I owe a lot to my beloved wife **Mrs. Parvatham** and my lovely daughter **Ms. Saraswathi** who have sacrificed much for making this endeavour a success.

#### CHAPTER I INTRODUCTION

Nature has bestowed varied agro-climatic conditions enabling our country to grow vegetables from sea level to snow line. Further, concerted efforts made in vegetable research and development have resulted in the development of more than 200 improved varieties / hybrids and a number of improved production and protection technologies in India. However, productivity of different vegetables in our country is comparatively lower than the Worlds average productivity. There is a tremendous strength in the vegetables to realize the high productivity per se with the proven technology (Kalloo and Pandey, 2002).

Ironically, in our country maximum emphasis has been given to important vegetables like tomato, brinjal, chillies etc., without giving much importance to indigenous vegetables like coccinia and gourds, which are our pride, inseparable from our custom and socio-cultural activities. They are under utilized and under-exploited vegetables (Indira and Peter, 1988). Here, great scope exists to enhance the area and productivity of these vegetables, which need concerted efforts in research strategies both at national and state levels. Though number of indigenous vegetables are grown in different parts the country, their recent statistical data is not available at national level, that indicates the attention we paid towards these vegetables.

Now this is the time to take up such crops and generate research information and promote these crops on the farmer's field and make awareness about benefits of indigenous vegetable among consumers.

One such important vegetable, indigenous to Asian sub tropic is *Benincasa hispida* (thunp) Cogn. which is a only cultivated species comes under genous *Benincasa* commonly known as ash gourd (Petha). Other names of this vegetable are wax gourd, white pumpkin and Chinese preserving melon (Nayar and More, 1998). It has Sanskrit equivalent 'Kooshmanda' as well as Persian 'Pagadaba' which signify

its antiquity (Varghese, 1973). The fruit has good source of nutrients and their nutritive values estimated by Esquinas-Alcazar and Gulick (1983) are as given below

Water Protein Fat		Carbohydrate	Vita	ımins (IU	()		Miner	als (mg	g)		
(g)	(g)	(g)		Vit A	B1	B2	C	Ca	Fe	Mg	P
96	0.2	0.1	3.5	Tr	0.02	0.5	14	14	0.4	16	7

(in 100 g edible matured fruits)

Besides its good source of nutrition, it has medicinal properties in fruits and seeds. It has been reported widely in pharmacopocias (book containing list of medicinal drugs and directions for their use). According to Ayurveda the fruit of ash gourd is laxative (helps bowels to empty), diuretic (increase flow of urine) and aphrodisiac (arouse sexual drive). It cures urinary infection, biliousness (bile attack) and certain blood diseases. The seed oil of ash gourd is considered as a cooling medicine. After incineration (burning) of the rind, the ash is applied to painful wounds. An enzyme extracted from ash gourd juice is used to treat variety of ailments in ayurvedic and naturopathy systems of medicine (Ramesh et al., 1989). The fruit may be eaten raw or cooked as vegetable marrow or candy. In our society, ash gourd fruit is considered as worship material used extensively for ceremonies, functions etc. It has wide adaptability and is cultivated in almost every part of India and all other tropical countries. Developing countries alone contribute about 50 per cent of the world production of major cucurbitaceous crops (Esquinas-Alcazar and Gulick, 1983). ash gourd has been reported to have been grown as vegetable in China since 500 AD (Ng, 1993). In India, it is grown predominantly in the states of Andhra Pradesh, Tamil Nadu, Karnataka, Maharastra and Kerala. As per 1997 statistical report, ash gourd has been cultivated in an area of 2497 ha with a total production and productivity of 15326 Million tonnes/year, 6.13 tonnes/ha, respectively (Sidhu, 1998) which is very low, if we compare other countries growing cucurbitaceous vegetables.

In Tamil Nadu, it is being cultivated in an area of 336 hectares with 6048 tonnes of production and 18 tonnes/ha of productivity (Anon. 2001). Coimbatore district alone contribute about 50% of total area (183 ha) and production (3294 tonnes) in Tamil Nadu as per the database (2000-2001) published by Tamil Nadu Horticulture Department. It is interesting that the use of Ash gourd fruit has increased in recent times and demand for quality seeds is overwhelming.

Availability of quality seed is most important for increasing the vegetable production and productivity. Vegetable growers recognize the quality seed of improved varieties as the most strategic resource for higher and better vegetable yield. Several reports are available with ash gourd production technology, which is entirely, differ from production of quality seed. Quality seed production needs extra effort in cultural practices, harvesting and storage etc. Production technology for seed, varies from location to location and varieties to varieties.

The growth of plant and seed production is strongly influenced by genetic factors and environmental conditions. These factors and their relationship with varietal adoptability in any given locality (Chadha, 2001) are the deciding factors for the better performance of the crop. Emphasis should always be laid on those factors, which contribute to and affect seed quality e.g. harvesting and post harvest operations. For achieving successful seed production, intricacies of the factors that create problems need be addressed for the crop in question.

Excessive production of staminate flowers and low number of pistillate flowers are the main contributors of decreased yield in cucurbits. These can be overcome if the sex ratio is maintained at an optimum level. This may be achieved by adjusting the sowing season (Kamalanathan and Thamburaj, 1970). As we need dry weather condition for seed crop during development and desiccation, raising crop during these season vis-à-vis seed quality must be studied under prevailing weather condition.

For good quality seed, fruits must mature on the vine. Premature or late harvesting of fruits results in reduced germination and recovery. Since fruits are mature sequentially, the growth cycle of the seed crop is appreciably longer than that for the fresh product. Hence, the seasonal requirements for seed production must be timed accordingly. By studying the seed development and maturation of a crop / varieties, we can plan for the cropping system, which suits either short or longer (Doijode and Sulladmath, 1982) period of cultivation.

The physiology of seed development immediately after fertilization, the seed becomes the primary recipient (sink) of the assimilates of the plant. As growth proceeds a seed typically undergoes three physiological stages. The first stage, in which approximately 80% of the growth occur, is characterized by numerous cell divisions and elongation as seed weight increases because of the supply of nutrients from the mother plant through the funiculus. The second stage is a period, when the funiculus degenerates and the seed is separated from the mother plant. At the time that this process is completed the seed possess its maximum dry weight. This period is defined as physiological maturity and is the time at which the seed is at its maximum quality level. Thereafter, the seed undergoes desiccation, which is the third stage. The desiccation period is a time in which the seed is influenced by a variety of weather conditions and when the seed is functionally stored on the plant. Timing of seed harvest has a major effect on seed quality since not all seeds will be at the same stage of maturity. Thus a knowledge of the environmental influence on seed development and quality is important in understanding seed production (Miller et al., 2001).

After fertilization seeds proceed through embryogenesis, maturation and desiccation (Bewley and Black, 1994), and at each stage different biochemical and physiological changes occur which will eventually accommodate germination and post germinative growth. Seed development is affected by a variety of factors

including genetic, physiological and environmental elements and the changes, which occur, cannot be correlated simply with time. The identification of distinct morphological and physiological characteristics is an important step in the study of seed development and maturation.

Seed production in the family of cucurbitaceae is more complicated than in dry seeded grain crops because in later, seeds mature within moist fruit and often held at high moisture content for several weeks before harvest. Variation in performance of commercial lots of cucurbits seed has been attributed to combining many individual fruit from different stages of development into a single lot (Oluoch and Welbaum, 1996).

Seed development in fleshy – fruited species has received less research attention than in dry seeded grain crops (Welbaum, 1999). External environment can disturb association of fruit and seed maturity and external signs of fruit maturity which serve to determine harvest date for fresh consumption or seed production in cucurbit crops (Nerson, 1991). Production of high quality seeds of cucurbit crops depends on harvesting the fruits at the proper stage of maturity as well as on proper extraction and storage procedures (Nerson and Paris, 1988). Such studies in ash gourd are still lacking. Therefore, precise information on optimum stage of harvest relates to season, varieties will enable the seed producer to harvest the fruit in time. To find out the correct stage of fruit harvest, it is essential to know the physio-biochemical changes that occur during the development and maturation of seeds (Balakrishnan *et al.*, 1986). Recently evolved techniques like Image analysis and electrophoresis evolved mainly for varietal identification can also be used to judge the seed maturity.

The present day tendency to apply simples, non-invasive technique has stimulated the development of visual image analysis (Saperstein, 1996). Researchers from across the world emphasizing integrated approach of combining morphological examination of seeds, seedling and matured plant for identification and characterization of varieties under DUS testing for award and implementation of breeders rights. The falling cost of electronic components now makes it practical to construct an inexpensive computer based system for the acquisition and analysis of images of dried seeds which may be included with other method for integrated approach (Travis and Draper, 1985).

Growth pattern of plant parts and evaluation of disease infection can also be done using image analysis system (Keefe and Draper, 1988 and Shahin *et al.* (1999) This technique is used most often based on the measurement of geometric properties. It should be possible to trace seed, embryo morphological changes using this system which can be correlated with other parameters in deciding the maturity status.

Reference point other than time after pollination is essential for experimenters to be able to compare data on seed development. Examination of seed structure and histo-chemistry of seed coat and cotyledon are also one of the option to be explored. which also can be correlated with morphological features recorded from other experiments. According to Jing *et al.* (2000) except cotyledon, changes in dry weight accumulation of seed coat and embryonic axis are not substantial during growth and development of cucurbits. Hence, ultra- structural changes in seed coat and embryo will be useful for maturity indication rather than drymatter accumulation, which may be applicable to ash gourd, a member of cucurbitaceae family.

Most of the studies on the development of crop seeds were restricted to the measurement of drymatter, protein accumulation and other constitutional changes, but storage protein synthesis in developing seeds of cucurbitaceous. Indian genotypes using electrophoretic system had not been attempted so for. Such studies are necessary for correlation. The results from this type of investigation will form the basis for further exploration.

Among various external and internal factors, seed development and seed position are two intrinsic factors that determine seed performance. The quality of seed in fleshy-fruited species is enhanced when they acquire maximum dry weight (Demir and Ellis, 1992). Fruit development and seed maturation may occur independently (Liu *et al.*, 1997). Thus seed harvested at the same time can be at different developmental stages.

Seed performance is also associated with seed position. The position of a seed is determined by the position of the ovules within the fruit and fruit positions on the mother plant. Based on studies in species with a linear arrangement of ovules within the ovary such as legumes and cucurbits, seed position effects may involve male gametophytic selection (Stephenson, 1992). To get successful seed performance the combined effect of seed development and seed position has to be studied (Demir and Ellis, 1992). In fleshy fruits, the seeds continue to develop and mature in the fleshy fruits until they get extracted from fruits (Ahmed *et al.*, 1987). To achieve uniform seed germination and seedling growth, developing seeds must be allowed for post ripening and drymatter accumulation beyond certain period. Hence, post harvest ripening in the form of fruit storage must be standardized for varieties per se in order to get uniform germination and seedling growth.

Physiological maturation can also continue while seeds are in the dry state although at a slower rate. In dry seeds, the process is called after ripening and the duration required to achieve maximum vigour varies with storage. Therefore cucurbit seed quality actually improved in storage condition and locality (Welbaum and Bradford, 1991). According to Barbedo *et al.* (1999) cucurbit seeds subjected to fruit storage after post harvest ripening maintained higher germination and seedling growth. Every body knows pretty well that ash gourd fruits can be stored for long time. How does it affects the seed quality, if doesn't, how long we can keep fruit under atmospheric room temperature? These questions need to be answered.

Fermentation of cucurbit seed is a common practice for facilitating the separation of seeds from the surrounding pulp (Whitaker and Davis, 1962). There are some problems in open fermentation method. One of the main problem is mould growth over fermentation material which may spoil entire content, cause discolouration of seeds and make them unfit for sowing. According to Nerson *et al.* (1985) washing and drying of extracted seeds have an important influence on germinability. Many of the experiments have been attempted to through light on these aspects, which covers mostly cucumber grown under controlled condition. Informations are scantly in ash gourd especially with regard to seed production. Since it is adopted to tropical condition it behaves independently unlike other cucurbitaceous vegetable grown in western countries, which has large patronage by majority of scientific workers.

CO 2 and Kerala local are currently popular and ruling varieties of ash gourd in Tamil Nadu and Kerala respectively. Tamil Nadu Agricultural University, Coimbatore has released the former in the year 1980 which is known for its good pulp content and cultivated all over Tamil Nadu (Sundararajan and Muthukrishnan, 1982). The latter was identified as promising selection suitable for central and South Kerala (Indira and Peter, 1988). The variety Kerala local was released during 2000 by Kerala Agricultural University. To study the effect of season in a given locality, the treatments were imposed in these two varieties to emanate comparison among these two.

To address some of the practical difficulties faced by the seed producers and farmers with respect to fruit and seed maturation and post harvest technologies of seed, the present investigation were carried out in CO 2 and kerala local of ash gourd with following objectives.

❖ To document the flowering behaviour as influenced by season and varieties.

- ❖ To assess the changes during the development and maturation of fruits and seeds due to varieties and season using physical, physiological, biochemical, electrophoretic and histological methods.
- ❖ To study the influence of fruit and seed position on seed quality.
- ❖ To find out the effect of fruit storage on seed viability.
- ❖ To standardize a suitable seed extraction, separation washing and drying method for large scale adoption.
- ❖ To test verify the best method of seed extraction and drying at seed producers unit.

#### CHAPTER II REVIEW OF LITERATURE

*Benincasa hispida* (Thunb.) Cogn. (2n=24) has great potential in curing number of ailments in human being. Use of *Cucumis* and *Benincasa* as vegetables and salad dish has increased in recent times probably, due to its cooling effect, as world is experiencing unprecedented temperature rise due to the climate change. Besides their vegetable use, fruits are used extensively in indigenous medicine like, unani and ayurveda. There is a tremendous demand for quality seed and huge gap exists between production and supply due to low productivity of this vegetable. Hence, to increase the productivity of this crop, proven technologies must reach to the farmers. In this context research works on this under-exploited crop is very much appropriate.

Very few workers had concentrated on seed technological aspects of tropical-cucurbitaceous vegetables in general and ash gourd in particular. Since, it is predominantly cultivated in Asia, it is difficult to get more information about this vegetable from the literature published elsewhere. Hence, available informations related to the present topic of research works are reviewed with major emphasis to vegetables.

The reviews were broadly divided into the following topics and presented chronologically.

- 2.1. Flowering behaviour influenced by season and varieties
- 2.2. Fruit and seed development and maturation
  - 2.2.1. Physical changes in fruit and seed
  - 2.2.2. Application of image analysis in seed technological studies
  - 2.2.3. Physiological changes in seed during development and maturity
  - 2.2.4. Histological studies

- 2.2.5. Biochemical changes and gel electrophoresis for storage protein
- 2.3. Influence of fruit and seed position on seed quality
- 2.4. Fruit storage on seed quality
- 2.5. Seed extraction

# 2.1. CHANGE OF FLOWERING PATTERN AND SEED GROWTH CHARACTERS DUE TO SEASON

According to Sharma and Nath (1971) in melon, the sex ratio is narrower in the rainy season than in the summer season. Further they have reported that sex ratio decreased with a rise of temperature during the summer (28.9 to 34°C) together with fall of RH from 48 to 31%.

Kamalanathan *et al.* (1972) obtained profuse flowering in the cucurbitaceous vegetables sown during the period of June – August when compared to summer season.

The duration of vegetative phase and the time of season at which seed development commenced, were both found to be important determinants of seed yield (Thurling and Das, 1980).

Boyle *et al.* (1986) has reported seasonal variation in vegetative and reproductive development in zinnia. Satisfactory performance in terms of days to flowering and other parameters were obtained when seed sowing was taken up in winter season.

Study on time of sowing on cucumber seed production by Sitaram *et al.* (1989) revealed that 30<sup>th</sup> January sowing was adjusted best for getting good quality seeds with best seedling vigour.

Significant differences observed among different cultivars for the traits namely, days to first flowering, length of fruit, diameter of fruit and number of seeds per fruit in pointed gourd as per the report by Singh (1991).

Extreme environmental condition causes no uniformity in seed and fruit maturity in muskmelon (Winsor *et al.*, 1987). External factors can disturb association of fruit and seed maturity and external signs of fruit maturity which serve to determine harvest date for fresh consumption or seed production in cucurbits (Nerson, 1991).

An increase in temperature from 20/10° to 30/20°C found to reduce mean onion seed dry weight from 2.90 to 2.55 mg as a result of a reduction in the duration of seed growth from 80 to 55 days (Gray *et al.*, 1992).

Sex mechanism in cucurbits is controlled by genetic, physiological and environmental factors (More and Seshadri, 1998).

Significant differences in days to male, female and number of female flowers were recorded in muskmelon grown in two environments with two temperature regimes in a glass house experiments (Ventura and Meudlinger, 1998).

Bottle gourd grown in three season *viz.*, summer, winter and rainy exhibited differences in flowering characters namely early yield, days to anthesis, node number in which first female appeared and fruit harvest (Maurya and Singh, 1998).

Satisfactory flowering and pod production with optimum seed quality were obtained in two okra varieties cultivated in the March sowing under Mediterranean region (Passam *et al.*, 1998).

Sirohi and Yayasani (2001) observed great variation among different genotypes of pumpkin for flowering and fruiting pattern.

#### 2.2. SEED DEVELOPMENT AND MATURATION

#### 2.2.1. Physical changes in fruit and seed

Rapid increase in diameter of the fruit of *Cucumis melo* was observed on 30 days after anthesis (Masuda and Kodera, 1953).

Harrington (1959) observed an increase in 100 seed weight upto 37 days after anthesis in muskmelon and no increase in number of seeds during fruit development.

Showalter (1961) had confirmed that in watermelon, the fruit after reaching the maximum weight, started loosing weight accompanied by loss in moisture content and this dehydration was an inherent phase of development.

McGlasson and Pratt (1963) recorded maximum value of seed size on 20<sup>th</sup> day after anthesis in *Cucumis melo*.

Hammet and Malithong (1971) stated that in cucumber, a linear fruit growth was slow during initial four days and reached maximum on 18<sup>th</sup> day after flowering.

Pratt *et al.* (1977) observed attainment of maximum fruit length on 30<sup>th</sup> day in muskmelon.

Varatharajan (1979) observed highest weight fruit at 42 days after anthesis in ribbed gourd cv. CO 1.

Chandrasekaran (1979) reported that peak fruit weight reached 24 days after anthesis in cv. Coimbatore long bitter gourd.

Low, peak and declining trend of moisture content was reported in phase I, II and III of fruit development in *Cucurbita pepo* (Culpepper, 1973).

Seed water content during the development of muskmelon fruit declined to 42% between 10 and 35 days after anthesis and declined more slowly to 35% at 50 DAA (Adams and Rinne, 1980).

Melon seeds mature and remain within a relatively moist environment where desiccation is prevented or delayed until after fruit senescence (Barlow *et al.*, 1980).

Muskmelon seeds posses atleast three developmental stages. In the first, rapid cell division begins after fertilization and embryonic axis and related seed tissues are formed. The second phase is characterized by expansion and accumulation of reserve materials. During the third phase metabolism is reduced, as water is lost from the quiescent dormant seed until metabolic systems are reactivated by imbibition (Welbaum and Bradford, 1989).

In some cucurbits, embryos develop the ability to germinate sequentially well before accumulation of maximum dry weight, which occurs roughly 35 DAA. At 25 to 30 DAA roughly 40% of epicotyls and roots grow, while remaining 60% showed expansion. The percentage germination of intact seeds was <50% at mass maturity. Greater than 95% viability was not obtained by newly harvested muskmelon seeds until 45 DAA (Welbaum and Bradford, 1989).

Germinability, vigour and desiccation tolerance of muskmelon seeds were reported in relation to changes in seed water content during development within fresh fruit by Welbaum and Bradford (1989). They obtained maximum germination and other parameters at 45 Days after anthesis.

Changes in fruit morphological characters *viz.*, fruit, seed cavity diameter, colour, size and shape and seed parameters namely fresh weight, during fruit growth and maturation of melon cultivars were reported by Miccolis and Saltveit (1991) in order to predict harvestable maturity.

Leek (*Allium porrum* L.) attained maximum seed growth rate and moisture content at 115 days after anthesis. Maximum germination was set only after fall in moisture content from 60% to 20-30% as reported by Gray *et al.* (1992).

Changes in tomato seed quality were studied by Demir and Ellis (1992) and reported that the end of the seed filling period occurred 35-41 days after anthesis and maximum seed quality were attained at the end of seed filling period and begin to decline immediately thereafter. Whereas, in Okra mass maturity of seed occurred

52 days after anthesis as reported by Demir (1994). Seed moisture content at this maturity was around 71%.

Anderson (1985) reported that maximum rate of increase in weight of *Cucurbita pepo* occurred between 11 days after pollination and 11 days previous to beginning of the ripening period.

Seed production in the family cucurbitaceous is more complicated than in dry seeded grain crops because seeds mature within moist fruit and often held at high moisture content for several weeks before harvest (Welbaum, 1999).

In majority of melons, germinability of seed, starts during 35 DAA, peaks at 65 DAA and decrease afterwards (Welbaum *et al.*, 1999).

In cucurbits, seed cavity volume to fruit volume ratio decreased with increasing length of the fruit. Pumpkin had the lowest ratio of seed yield to fruit weight (Nerson *et al.*, 2000).

Developing cucurbits seeds from 4 to 9 weeks after anthesis showed marked increase in embryo weight, size, volume and oil content as per the report by Ockenden *et al.* (2001).

Bhattacharya *et al.* (2002) divided the entire reproductive phase into 10 stages and categorized six stages of seed development from 5<sup>th</sup> stage onwards which starts from visible embryo (1), rapid growth of seed and embryo (2-7), filling (8), maturation (9) and ended with mature seeds about to be shed from fruits (6). They further studied the maturity index to determine the optimal time of seed collection.

Fruit weight, length and number of seeds of developing okra cv. Parbhani kranti fruit were studied to determine the optimum stage of seed harvest by Samnotra *et al.* (2002) and they reported that the difference of above growth parameters in spring summer and rainy season crops.

#### 2.2.2. Application of image analysis system in seed technological studies

The application of image analysis in agriculture for varietal identification is reported by several authors (Price and Osborne, 1990; Heijden and Vander, 1990; Heijden *et al.*, 1989; Kranzler, 1985).

Image analysis lends itself for computerization and thus enables the processing of large amounts of material for examination in a relatively short time (Baun and Bailey, 1987).

Machine vision has been utilised for cultivar description, characterization and identification of varieties (Draper and Travis, 1984) using seeds and plant parts.

Van de Vooren *et al.*, (1991) used image analysis for variety testing of mushroom and made distinction between 80% of the cultivars at 1% significant level.

Travis and Draper (1985) separated 49 different crops and weed species on the basis of shape in combination with length factor using image-processing system.

Myers and Edsall (1989) were able to determine the Australian wheat varieties to an acceptable accuracy using the machine vision procedure of discriminant analysis.

Maccormac *et al.* (1990) explained the difficulty in measuring five roots produced by seedlings by image analysis system and attempted modifications in it.

Finch – Savage (1986) measured seedling growth of some vegetables using image analysis system and reported positive relationship of the results with seed vigour and field performance.

Van de Vooren and Heijden (1993) performed measurement of pod length and width using image analysis and compared with manual measurement in French beans.

Mudzana *et al.* (1995) compared manual evaluation with image analysis system for plant morphology.

Using machine vision, Shatadal *et al.* (1995) identified other crop seeds, which looks close to test crop seeds.

Dehghan – Shoar *et al.* (1998) discriminated several varieties of lucerne by capturing images of seed morphological characters and recommended this method for seed certification.

Keefee (1999) utilised the image analysis system for measuring linseed descriptors and able to distinguish all the tested varieties.

Jayas *et al.* (1999) developed automatic seed positioning systems for placing individual cereal seed under a camera for image acquisition.

Image analysis system was used by Aquila *et al.* (2000) to measure area, perimeter, width and length of white cabbage seeds in order to monitor changes in seed physical parameters during imbibition and suggested that image analysis techniques have high potential in seed biology studies.

Sahoo *et al.* (2000) reported varietal discrimination of sunflower seeds using machine image approach.

Change of lentil, uniformity and discolouration due to developmental variation in seed can be tracked using machine vision (Shahin and Symons, 2003).

#### 2.2.3. Physiological changes in seed during development and maturity

To ensure fully mature seed with high germination, muskmelon fruit should be harvested only when it shows good netting and the stem breaks away from the fruit for more than half of its circumference (Harrington, 1959).

In tomato maximum germination attained 10 days before mass maturity, 63 months of subsequent storage, seeds harvested 10 days after mass maturity showed improved germination as reported by Doijode (1983).

Delaying the planting date of soybean cultivars led to delay in harvestable maturity improved seed germination, vigour and reduced level of infection by phomopsis disease (Tekrony *et al.*, 1984).

In papaya cv. CO2, maximum germinability correlated with seed size, fruit weight and volume were observed at 130 days after anthesis (Balakrishnan *et al.*, 1986).

Kanwar and Saimbhi (1987) could obtain matured seeds with high germination and seed weight at 35 days after anthesis in Okra under Punjab condition.

Kwon and Bradford (1987) reported that maximum viability and vigour occurred in tomato 15 days after mass maturity.

In faba bean there was a substantial decline in viability and an increase in seedling abnormalities when harvest was delayed beyond the optimal moisture content for harvest (Ellis *et al.*, 1987).

Denuff and Rachidian (1988) observed disruption of vascular connection between pod and mother plant at the time physiological maturity in pea.

Chamma *et al.* (1980) could obtain maximum seed quality from the resultant pods harvested when green pods no longer found on the plant in *Phaseolus vulgaris*.

On set of germination starts even in premature stage during development of *Quercus robur* L. which showed variation in germination rate due to shedding date (Finch-savage, 1992).

The on set of both germinability and desiccation tolerance occurred either just before or at mass maturity (53 DAA) when moisture content reached 51-53% in pepper during seed development as reported by Demir and Ellis (1992).

Sundralingam (1995) obtained good quality seeds harvested at physiological maturity, which occurred seventh week after anthesis.

Naik *et al.* (1996) cautioned that harvest of capsicum fruits beyond physiological maturity affects the seed quality.

Gurusamy (1996) reported that cauliflower seeds could be harvested within a week after reaching physiological maturity.

Viability of cucumber seeds were assessed by Tetrazolium test, indicating the initial viability of fresh seed as 93% and reported wide difference between fresh seed germination and viability result from TZ test (Suryawanshi *et al.*, 1996).

Baruah and Paul (1997) concluded that okra pods could be harvested 35 to 42 days after flowering to obtain good quality seeds.

Reddy and Rolston (1997) indicated that yield and quality of coriander seed was maximum at 37 to 42 days after peak flowering.

Ren and Bewley (1998) have made discrete continuous stages in seed development of Chinese cabbage as pre embryo, pre cotyledon, early cotyledon, enlargement 1, enlargement 2, filling and maturation 1, 2 and 3, desiccation and Quiescence.

Barbedo *et al.* (1999) found that cucumber seeds maintained higher germination when harvested at 30-35 days after flowering.

Menaka (2000) obtained maximum germination and seedling vigour from the seeds harvested at 25 days after anthesis when physiological maturity coincided.

Cent per cent viability and germination were reported in the tea seeds perior to shedding which occur approximately 15-16 months after anthesis. Whereas, maximum rate of drymatter accumulation was observed after approximately 12-13 months after anthesis (Bhattacharya *et al.*, 2002).

Muasya *et al.* (2002) studied the maturity index of three classes *viz.*, early, medium and late formed pods of common bean and concluded that development of final red purple colour pattern was reasonable indicator of physiological maturity and depending upon earliness pods tend to reach physiological maturity early.

#### 2.2.4. Histological studies

Vaughan (1959) has illustrated the testa coat structure of *Brassica campestris* after sectioning to know the developmental stages.

Endosperm development has been investigated in three members of cucurbitaceae by Chopra and Seth (1977) and reported that during development the central cells of the cellular endosperm and their nuclei enlarge consistently and remains permanently coenocytic.

Welbaum and Bradford (1990) reported microscope study on seed embryo, testa endocarp and perisperm envelope to know the permeability characteristics of the tissues surrounding muskmelon embryos during development from 20 to 65 days after anthesis.

Seed coat structures and histochemistry of *Abelmoschus esculentus* were studied using scanning microscope by Valenti *et al.* (1992) to know the seed coat related dormancy.

Hilhorst and Downie (1995) reported that due to the presence of four or five layers thickness of testa of wild type tomato seed, germination gets delayed while cultivars having thin one cell-layer thickness favours early germination.

Kuang *et al.* (1996) studied the embryo structure of different developmental stages in *Arabidopsis* plants grown under space flight condition and found that starch and protein reserve deposition in the embryos during tissue differentiation in a manner similar to that in ground control and seed coat was developing properly.

Ren and Bewley (1998) are in opinion that for experimenters to be able to compare data on seed development, reference point other than time after pollination are essential. They examined white cabbage testa structure at different developmental stages and observed growth of epidermal and the sub-epidermal cells, thickening of palisade cells. In mature dry seeds, the testa has become hard and all layers are crushed into non-structured cells.

Sugiyama and Morishita (2002) studied the development of watermelon seed from 0 to 20 days after pollination and examined the development of embryo, endosperm and seed coat structures.

Dangou *et al.* (2002) studied the histological and biochemical characteristics of cocoa endosperm and determined at different stages of seed development and observed that endosperm degenerate as developmental stages advance.

#### 2.2.5. Biochemical changes during seed development maturation

Chittiraichelvan and Shanmugavelu (1977) opined that papaya seeds attained maximum accumulation of seed protein at 135<sup>th</sup> day after anthesis.

Crouch and Sussex (1981) detected both 17s, 12s protein during early embryo development and added that accumulation of the 17s protein stopped when the water content of embryo began to decline, but accumulation of the 12s protein continued until seed maturity in *Brassica napus* L.

Immature seeds *Phaseolus vulgaris* failed to germinate without desiccation which is associated with protein synthesis and desiccation plays a role in permanently suppressing developmental protein synthesis and in inducing germination protein synthesis (Dasgupta and Bewley, 1982).

The synthesis of soluble protein in developing castor bean endosperm (in the 30-33 kDa range) commences by 20 days and continues upto 50 days. Other protein (eg, 45 and 46 kDa polypeptides) are synthesized exclusively during late maturation (40 to 50 days of development) (Kermode *et al.*, 1986).

Changes in fruit weight, length girth along with biochemical changes in chilli were studied during fruit and seed development in chilli by Rama Rao *et al.*, (1990) and reported that peak seed quality achieved when pod colour changed to yellow.

Blackman *et al.* (1991) indicated that desiccation of developing seeds was characterized by the accumulation of a set of mRNA and related proteins called late embryogenic Abundant (LEA) proteins in the desiccation stage of pea.

Sreenivasulu and Amritphale (2000) reported changes in protein composition in cellular membranes of seed parts in cucumber seeds treated with ethanol during imbibition.

According to Dasgupta and Mandal (1993) the major classes of storage protein (12s and 2s) were appreciably synthesized two weeks after flowering during seed development in cauliflower.

#### 2.3. POSITION OF FRUIT AND SEED ON SEED QUALITY

Among various external and internal factors, seed development and seed position are the two intrinsic factors that determine seed performance (Rees, 1997). Initially seed performance is determined during seed development and maturation. Fruit development and seed maturation may occur independently. Thus, seeds harvested at the same time can be at different developmental stages.

Chauhan and Bhandari (1971) reported that Okra seeds from mid portion of the pod showed early maturity (27 days after anthesis) compared to those from apical and stem end portions (30 DAA).

The seeds produced at different position would influence not only the yield but also the quality of the seeds produced. Seeds harvested from central umbels produced more productive plants in carrot (Shevtosva and Korol, 1976).

In cucumber, seeds extracted from fruits emerged early on the lower internodes of main stem found to show best seed quality as reported by Abramova (1980).

Varis and George (1981) stated that seeds harvested from different trusses showed no marked effects on germination and seedling vigour in tomato.

Novikov (1983) observed the decline of 1000 seed weight in tomato from the first to the third harvesting date due to an increase in the proportion of small seeds.

The best seed quality and yield were produced in okra by the pods on the third and fourth nodes, where pods mature early (Prabhakar *et al.*, 1985).

In chilli, seeds form basal region of the fruit exhibited higher germination and seedling vigour than those from tip and middle positions as reported by Doijode (1990).

Seed position inside the developing muskmelon fruit likely affects seed development in similar to that of fruit position (Stephenson, 1992).

Vijayakumar *et al.* (1994) reported that bittergourd seeds developed at proximal 1/3<sup>rd</sup> portion of fruit registered higher seed weight, embryo weight and germination percentage than middle and distal end.

Variation in performance of commercial lots of muskmelon seed has been attributed to combining many individual fruit from different stages of development into a single lot (Oluoch and Welbaum, 1996).

Development of seeds originated from early and late flowers on main receme of *Brassica* was studied by Nanda *et al.* (1996) and they reported that the rate of drymatter accumulation remained higher in late-formed pods when compared to earlier seeds. Seeds in upper positioned pods had low per cent oil.

According to Sivasubramanian (1996), seeds collected from middle portion of the fruit recorded maximum vigour index than proximal and distal end in moringa.

Verma *et al.* (1998) reported that as the order of capsule position increase, there was decrease in length of capsule, germination and seed weight due to source and sink relationship within the plant and seeds / capsule was reported to be high in fourth node capsule position in okra.

Seeds from Siliqua positioned in proximal end between 61-80<sup>th</sup> node found to showed maximum germination which reflected in other parameters like protein oil and drymass as well (Still, 1999) in cabbage.

Doijode (2000) stated that in long fruited brinjal, seeds borne in different position within the fruit showed significant variation during storage in terms of crop and seedling growth.

Doijode (2000) conducted experiment to study the influence of position of seeds in the squash fruit and reported that early emergence, better germination and seedling growth was observed in the seeds extracted from first quarters of the fruit when compared to other position towards pedicel end.

Andrea *et al.* (2001) reported that the total number of seeds in a spike was not influenced by the position of the spike on the stem in marjoram plant, which found to show uniform growth with contemporary seed maturation.

#### 2.4. POST HARVEST FRUIT STORAGE

Harington (1959) reported that harvesting fruit at correct maturity and subject to pre-extraction storage of harvested fruits resulted improved germination in muskmelon.

Water loss during storage has been reported as a possible factor influencing storage life of wetted muskmelon fruit as reported by Kasmire (1978).

Storage of cucurbit seeds for several weeks or months has been found beneficial for germination improvement (Watts, 1978).

Young (1979) recorded increased 100 seed weight, germination during fruit storage in mature and immature squash fruits after four months of storage.

Early harvest of fruit and subjecting them for post harvest ripening had improved the quality of seeds in cucumber as reported by Wallerstein *et al.* (1981).

Alvarenga *et al.* (1984) reported that harvesting fully ripen fruits of watermelon and subjecting them to storage resulted in poor quality of seeds due to high respiration and field damages as a result of delayed harvest.

High temperature during post harvest storage had detrimental effect on storage life of muskmelon (Hawker, 1985).

Cohen and Hicks (1986) reported that storage of melon fruits for the period of three weeks has no influence on soluble solid content when they harvested at full maturity and stored at 12-20°C temperature.

Priestley (1986) reported that post-harvest dormancy in muskmelon seeds could be removed by dry storage or after ripening.

Harvesting at optimum stage of fruit maturity and storage, not only minimize the loss of viability and vigour of seeds but also prevents the seeds from field damage due to insect pests and diseases and adverse environmental conditions (Ahmed *et al.*, 1987).

Seed quality of cucurbits can be improved by pre extraction storage of harvested fruits (Buriev, 1987).

Singh and Singh (1987) reported that fruit storage of summer squash resulted in numerical improvement in germination, seedling vigour and storage did not showed any beneficial effect on seed quality.

Physiological maturation can also continue while seeds are in dry state although at a slower rate. Cucurbit seed quality actually improved during storage (Welbaum and Bradford, 1991).

Pre-extraction storage of different cucurbits had a positive effect on germinability but was less effective than leaving the fruit on the vine for a comparable period of time (Nerson, 1991).

Among many cucurbit fruits melons are classified as climacteric because they exhibit large increases in respiration during ripening after harvest makes difficulties in storage (Abeles *et al.*, 1992).

A respiratory rise was observed with fruit ripening on the plant, but only after they abscised. The climacteric burst in respiration, which has been widely observed with ripened harvested fruit, may be an artifact of harvest and not a natural phenomena associated with ripening of climacteric fruit (Shellie and Saltveit, 1993).

Miccolis and Saltveit (1995) reported the decline in firmness and weight in muskmelon fruit storage when it was harvested at horticultural maturity and stored for three weeks at controlled condition. But soluble solids, external colour and flesh colour remained almost, constant during reported storage period.

Harvesting fruits at 50 days after anthesis and storing them for the period of 15 days resulted in superior seed quality *viz.*, germination, field emergence and vigour index when compared to freshly harvested cucumber fruits (Nandeesh *et al.*, 1995).

Zong *et al.* (1995) reported that immature fruit of bitter melon maintained post harvest quality better than fruit harvested at the fully developed green stage.

The pumpkin fruits harvested at full maturity reported to have a long storage life of one year under atmospheric ventilated room condition and they reach marketable maturity 75-180 days after sowing depending upon the variety and environmental conditions (Sharma and Lal, 1998).

Cucumber fruits harvested and stored in a store house at 5°C and 70 % RH for the period of 5 months and subjected to extraction showed decline in germination when they stored without drying to safer moisture content (Zeng *et al.*, 1998).

To get quality seed in cucurbits, fruits are allowed to mature on the plant itself or harvested and then allowed to ripen fully to attain seed maturity before extraction (Dadlani and More, 1998).

Bottle gourd fruits harvested at the natural death of the plants and shade stored for 57 days resulted in highest germination and vigour. The storage of the seeds in the

fruit was associated with the improvement of seed physiological quality at extraction (Bisognin *et al.*, 1999).

Barbedo *et al.* (1999) reported that seeds with high germination from either ripe (40-45 DAF) or immature (30-35 DAF) fruits of cucumber with 10 or 15 days of post harvest fruit storage maintained viability for a longer period.

Large sized matured fruits are reported to have good storability and bolder seeds which produce healthy and vigorous seedlings in ash gourd (Mini *et al.*, 2000).

Doijode (2000) conducted storage studies with winter squash and experimental result revealed that under ambient temperature seed could retained initial germination to two years afterwards, it reduced to 50 per cent during fourth year.

#### 2.5. SEED EXTRACTION

Interaction between fermentation and seed maturity on seed quality is effective if it is delayed (Whitaker and Davis, 1962).

Fermentation of cucumber seed for an extended period was reported to be harmful as reported by Nienhuis and Lower (1981).

Singh *et al.* (1985) evaluated durations of fermentation *viz.*, 24, 48 and 72 h using muskmelon fruits of three different maturity and result indicated that 24 h fermentation of fruits of full maturity showed improved germination.

Fermentation is beneficial for improving the rate of germination in watermelon and cucumber; simple washing is preferable over fermentation for melon and squash (Nerson *et al.*, 1985).

Mukhopadhyay and Chattopadhyay (1988) attempted seed extraction of pointed gourd using three concentrations (1%-3%) of HCl and result showed that 3% HCl found to boost seed germination compared to manual extraction.

Welbaum and Bradford (1989) reported that washing drying or washing followed by drying of fleshy-fruited immature watermelon seeds showed increase in seedling vigour as compared to fresh seeds, which received no treatment.

Oluoch and Welbaum (1996) concluded that seeds of musk melon subjected to extraction procedure namely washing vigorously for 3 h and dried to a moisture content of 3.3-5.7% had higher germination percentages, lower mean times to germination and greater resistant to controlled deterioration than unwashed seeds.

In dry extraction process (Squash, Pumpkin, Marrow and other Cucurbits) the seed is scooped out from the fruit and sun dried, the seed is then removed by rubbing over a sieve and inert matter is separated by winnowing or mowing (Dadlani and More, 1998).

Seed extraction by Willson (1999) reported that rubbing cocoa beans in saw dust or sand found to reduce the germination time for 7 days due to mechanical damage.

#### CHAPTER III

#### MATERIALS AND METHODS

# 3.1. MATERIALS

Genetically pure seeds of CO 2 and Kerala local (BH 21) of ash gourd were procured from Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore and Kerala Horticultural Development Board (KHDB), Palakad (Kerala), respectively and used for conducting this research work.

#### 3.2. METHODS

The research work consists of five experiments and a on farm trail as detailed below and methodologies followed for field as well as laboratory experiments are presented in this chapter.

- 3.2.1. Flowering behaviour as influenced by varieties and season
- 3.2.2. FRUIT AND SEED DEVELOPMENT AND MATURATION AS INFLUENCED BY VARIETIES AND SEASONS
- 3.2.3. Influence of fruit and seed position on seed quality
- 3.2.4. Effect of fruit storage on seed quality
- 3.2.5. Standardisation of a suitable seed extraction, separation washing and drying method
- **3.2.6.** On Farm Trial for test verification of the best method of seed extraction, separation washing and drying

Bulk seed crop was raised in two seasons viz., summer (January – June 2002) and rainy (July -December 2002) to study the seed development and maturation and other laboratory experiments. The flowering behaviour and visible descriptive growth and maturity symptoms of fruit and seed in the field were also assessed.

The study on the effect of fruit storage was carried out with fruits harvested from summer crop and investigations on fruit and seed position and standardisation of method of seed extraction, separation cleaning, washing and drying were performed in rainy season crop. The experiments were carried out at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. On farm trial was conducted at Kondamuthunur Village of Sathyamangalam taluk, Erode district of Tamil Nadu.

Summer crop was raised in the block No. 37A (Plate 1a) and rainy crop was raised in the field located (Plate1b) in front of Department of Seed Science and Technology. The soils of both the fields are moderately fertile and sandy loam with uniform properties.

Sowing was done in pits of 40cm<sup>3</sup> size adopting a spacing of 2.5 m either way. Before sowing, each pit was supplied with 20 kg of well decomposed farm yard manure and 100 g of 6:12:12 NPK mixture. Copious irrigation was given before the start of sowing to ensure settlement of soil mixture in the pits. All other cultural operations and plant protection measures were followed uniformly for both the seasons as per the technical guidance described in the crop production guide of Tamil Nadu Agricultural University.

After initial establishment of seedlings, less vigorous and off types plants were removed from the pits allowing only two vines per pit. The methodologies followed are furnished below.

## 3.2.1. Flowering behaviour as influenced by varieties and seasons

To document the flowering behaviour, ten plants from each variety in each season were chosen and the following observations were:

# 3.2.1.1. Days to male flower bud initiation to anthesis

First staminate (Plate 2a) bud appeared in first node of tendril side was tagged and duration taken for anthesis was recorded in days.

#### 3.2.1.2. Days to female flower bud initiation to anthesis

First pistillate bud (Plate 2a) appeared in first node of tendril side was tagged and duration taken for anthesis was recorded in days.

# 3.2.1.3. Days to first male flower initiation

Time taken for first staminate flower from sowing date to anthesis was recorded and expressed in days.

## 3.2.1.4. Days to first female flower initiation

Time taken for first pistillate flower from sowing date to anthesis was recorded and expressed in days.

# 3.2.1.5. Node number at which first male flower appeared

Node number, bearing first staminate flower was noted in the chosen plants and expressed as number.

# 3.2.1.6. Node number at which first female flower appeared

Node number, bearing first pistillate flower was noted in the chosen plants and expressed as number.

# 3.2.1.7. Sex ratio

The ratio of male to female flowers was recorded by counting the individual male and female flowers whenever they appeared during the entire crop duration in the selected plants and expressed as ratio of male to female

# 3.2.1.8. Number of fruits per vine

Total number of fruits per vine was recorded at the time of harvest in the selected plants and their average value was expressed in number.

After getting 50% flowering, the plants that were uniform in growth alone, were taken for recording further observations.

# 3.2.2. Fruit and seed development and maturation as influenced by varieties and seasons

Fifteen simultaneously developed pistillate flowers showing knob at the bottom (Plate 2a) of petals for each fruit to be harvested, were tagged at the time of anthesis (7-7.30 AM) as per the method described by McGlasson and Pratt (1963) for muskmelon, by noting the date of anthesis and the date on which the fruit has to be harvested to observe subsequent changes during fruit and seed development.

Accordingly, fifteen numbers with three replications of five fruits each were harvested at five days interval from the five to 80 days after anthesis for rainy season and 70 days for summer crop. The growth stages beyond 70 days after anthesis could not be studied in summer crop as there were no sufficient intact fruits and crop attained senescence earlier than rainy crop. Thus, from every stage, fifteen fruits were harvested for doing laboratory observations concerned with changes during growth and development. Same sample size and replications were followed for all the parameters pertaining to changes in fruit and seed. Changes made based on necessity were furnished in the appropriate sections.

Samples were extracted at 5-80, 20-80 and 30-80 days after anthesis with five days interval for investigating histology, Image analysis and seed characteristics, respectively. Summer crop was restricted to 70DAA for all the observations

Fruits, which showed deviation in development, were discarded and healthy fruits were alone taken for observations

#### **3.2.2.1.** Fruit weight

The whole fruits were weighed individually in a physical balance and average weight was recorded in g.

#### 3.2.2.2. Fruit circumference

The circumference of the fruit was measured by passing a thread along the centre of the perimeter and the length of the thread, which covered the entire fruit, was measured in centimetre after stretching.

# 3.2.2.3. Fruit length

The length of fruits was measured from the base (pedicel end) to the apex (stylar end) of the fruit excluding the stalk and expressed in cm.

# 3.2.2.4. Fruit diameter

Each fruit was cut into half along the longitudinal plane and the internal fruit characters were evaluated as per the method described by Miccolis and Saltveit, (1991) for fleshy fruited cucurbits

The whole centre portion covering skin, pulp and seed cavity was taken into account for measuring fruit diameter and measured value was expressed cm.

# 3.2.2.5. Thickness of the fruit pulp

The edible portion between peel (skin) to seed cavity was measured in the centre portion of the fruit and expressed in cm.

# 3.2.2.6. Seed cavity diameter

The diameter of the seed cavity was calculated by twice subtracting the pulp thickness from the fruit diameter and expressed as cm.

After evaluating fruit characteristics, the seeds were extracted manually and subjected to repeated washing for about five times with constant stirring (Nerson, 1991). Extra washings were given while separating immature seeds. The separated seeds were subjected to the following observations.

# 3.2.2.7. Number of well-filled good seeds (Krishna Prasad, 1980)

After extracting seeds from the fruit pulp, the entire seed contents were taken in the two litre plastic bucket and subjected to floatation technique to separate chaffy and immature seeds from well-filled good seeds. The water was added, three fourth of volume of container. The floating chaffy and immature seeds and other mucilaginous materials were removed and discarded. The well-filled good seeds settled in the bottom of bucket were collected and dried under room condition. Total number of well-filled good seeds was counted and expressed in number per fruit.

# 3.2.2.8. Fresh weight of seed

The extracted seeds from respective stages were placed in paper towels to remove external moisture and immediately weighed in an electronic balance to determine the fresh weight and expressed as g per fruit.

# 3.2.2.9. Dry weight of seed (Shivkumar et al., 2001)

After taking fresh weight, the whole seed extracted from each fruit of different stages was dried at 60°C for 36h in an oven and the dried seeds were weighed, after cooling using desiccator for 30 min. in an electronic balance to estimate dry weight of seed per fruit and expressed in g.

# 3.2.2.10. Moisture content of the seed (ISTA, 1999)

The moisture content of the seed was estimated by low constant temperature method by taking them in moisture bottle and placed in a hot air oven previously heated and maintained at 105°C for 16 h. After closing the mouth of the bottle with lids, they were rapidly transferred to a desiccator and allowed to cool down to a room temperature for 30 min. The seed moisture content was estimated by using the formula

Where,

 $M_1$  – Weight of the bottle and lid

M<sub>2</sub> – Weight of bottle, its lid and seed before drying

M<sub>3</sub> – Weight of bottle, its lid and seed after drying

# **3.2.2.11.** Hundred seed weight (ISTA, 1999)

After drying the seed to constant moisture content of ten per cent, the weight of eight replicates of 100 seeds each was taken in an electronic balance and expressed in g.

#### 3.2.2.12. Fruit to seed recovery

Fifteen fruits (three replicates of five fruits each) were selected at random from each stage of development and the seeds were extracted for estimating seed recovery. Before estimation, the moisture content of the seed was reduced to ten per cent and seed recovery was calculated using the following formula and mean value was expressed in per cent.

$$Seed \ recovery \ (\%) = ---- x \ 100$$
 
$$Total \ fruit \ weight$$

# 3.2.2.13. Cotyledon weight (Ockenden et al., 2001)

Thirty air-dried cotyledons with three replications of ten cotyledons each from all the development stages were weighed in the precision electronic balance and expressed in g.

# 3.2.2.14. Cotyledon thickness (Ockenden et al., 2001)

Thirty air-dried cotyledons with three replications of ten cotyledons each from all the development stages were measured with Vernier calipers in the centre of the flat cotyledon pair (Surface to surface) and expressed in mm.

# 3.2.2.15. Speed of germination (Maguire, 1962)

Four replicates of twenty-five seeds each were used to test the speed of germination of seeds from different stages (30 DAA on wards) of fruit development. Stages having 5 to 25 DAA of fruit age were discarded as they posses immature and small embryo. The seeds showing radicle protrusion more than 3.0 mm was counted daily from sixth day after sowing until fourteenth day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and result was expressed in number.

$$\label{eq:Speed of germination} \begin{aligned} Speed \ of \ germination = & \begin{matrix} X_1 & X_2\text{-}X_1 & Xn-X(n\text{-}1) \\ \hline Y_1 & Y_2 & Yn \end{matrix}$$

Where,

Xn = Per cent germination on n<sup>th</sup> day

Yn = Number of days from sowing to n<sup>th</sup> count.

# 3.2.2.16. Germination (ISTA, 1999)

Germination test was conducted using metal trays filled with sterilized sand medium. The germination room was maintained at  $25 \pm 5$ °C temperature with  $95 \pm 3$  per cent relative humidity. For each stage of development, four replicates of 25 seeds each were used. At the end of final count i.e. fourteenth day, the number of normal seedlings alone was counted and expressed in percentage.

# **3.2.2.17. Shoot length**

The distance between the collar and tip of the shoot was measured from ten normal seedlings and the mean value of shoot length was expressed in cm.

# **3.2.2.18.** Root length

Ten normal seedlings per replicate were taken at random from the germination test. The distance between the collar and the tip of the primary root was measured and the mean value was expressed in cm.

# 3.2.2.19. Dry weight of seedlings

The seedlings in which, the above measurements taken, were dried under shade and then kept in a hot air oven maintained at 85°C for 48h. Then, the samples were cooled in a desiccator for 30 min. and then weighed in an electronic balance and expressed in mg seedlings<sup>-10</sup>.

# 3.2.2.20. Vigour index (Abdul-Baki and Anderson, 1973)

The vigour index was calculated using the following formula and value was expressed in whole number.

Vigour index = Germination (%) x Mean length of seedlings in cm.

# 3.2.2.21. Viability evaluation by tetrazolim test

2, 3, 5- triphenyl tetrazolium chloride (TTC) test was employed at 0.25 per cent concentration buffered to Sorensons buffer. Twenty five seeds from each stage of development (30 DAA onwards) were taken in three replicates and soaked in water over night and the seed coat and their inner perisperm were removed carefully without causing any injury to the cotyledon. Then, the decoated seeds were soaked in three ml of TTC solution and kept for two hours in an incubator in darkness maintained at 40°C for staining. The stained and unstained seeds were subjected to evaluation. Viability was calculated based on staining pattern and reported in per cent.

#### 3.2.2.22. Oil content (Ockenden *et al.*, 2001)

Twenty-five numbers of dry decoated seed was ground in an iron mortar and transferred to an extraction thimble. The thimble was placed inside the soxhlet extractor to which sufficient quantity of petroleum ether (40-60°C) was added and heated for 6 h until 6-8 siphoning was completed. The remnant of petroleum ether was removed by heating (60°C) the bottle in an oven. The estimation was carried out in triplicate sample. The weight of oil content per seed was calculated and expressed in mg per seed.

# 3.2.2.23. Dehydrogenase enzyme activity (Kittock and Law, 1968)

The stained seeds were taken after estimating viability per centage (3.2.2.21) and thoroughly washed in water and transferred to a test tube containing ten ml of methyl cellosolve and allowed to stand for six hours at room temperature for extracting the red colour formazan. After complete extraction as indicated by the original white colour of the cotyledon, the optical density of colour development was determined with an ERMA photoelectric colorimeter (Model AE11) using the blue filter at 470 nm with methyl cellosolve as the blank.

# 3.2.2.24. Protein content (Alikhan and Youngs, 1973)

The protein content of seeds was estimated by calorimetric method. A sample of 100mg of powdered decoated seed was taken in a 50ml polyethylene screw cap bottle and 25 ml of 1N NaOH was added. The mixture was shaken for 30 min. at a wrist shaker to disperse the protein. The suspension of 10 ml was poured into a graduated test tube and used as blank to compensate the differences in amount of natural pigments extracted. To the remaining suspension in the bottle, 0.25 ml of 10 per cent copper sulphate was added and the bottle was shaken again for five minutes to develop the colour complex. The sample solution was then poured into a separate test tube along with its blank and left overnight to allow the dispersed material to settle down. After centrifugation for 10 min. 3000 rpm, the optical density of the clear supernatant solution was measured in Spectronic 20 using 620 nm filter with respective blank. From the mean optical density value, the protein per cent for each growth stage was calculated using the formula given below

Protein (%) = 
$$3.78+(61.6\times OD \text{ value})$$

# 3.2.2.25. Descriptive characteristics of fruits

At every stage of fruit development, using the criteria of ash coat development, pubescence, and desiccation in the pedicel etc., fruit descriptive changes were observed for the purpose of assessing maturity indices.

# 3.2.2.26. Descriptive characteristics of seeds

Developmental visible morphological changes viz., grooves in the seed coat, hardiness, attachment with placenta and desiccation symptoms etc., in all the stages of seeds were studied in the extracted representative seed sample from the fruits harvested for taking observation on fruit and seed characteristics reported above.

Minimal descriptors prescribed for ash gourd by Srivastava *et al.* (2001) were also recorded for CO2 and kerala local during the course of experiment and results are presented in the Appendix I.

# 3.2.2.27. Histological studies

The structural changes were studied by microtome sectioning of seed coat and embryo. The seed coat development was studied 20 DAA onwards and observed upto penultimate maturity of 80 DAA with ten days interval. Embryo development was studied during the ovule developmental stages viz., 5 to 80 with ten days interval. The methodology developed by Johanson (1960) was followed for microtome sectioning with necessary modifications. The details of microtome procedure followed are furnished below.

#### **Preparation of FFA solution**

FFA was prepared by mixing the following chemicals and used for fixation of specimens

Ethyl alcohol (95%) - 50 ml

Glacial acetic acid - 5 ml

Formalin - 10 ml

Distilled water - 35 ml

# **Fixation**

The materials were fixed in the fixative for 18 h and then stored in 70 per cent ethanol until dehydration process was started. Before fixing, dry mature seeds of different stages were put to imbibition in water for 12 h to soften the testa and allow easy penetration of fixatives (Davis *et al.*, 1988).

# **Dehydration**

Fixed materials were passed through different grades of tertiary butyl alcohol and ethanol as indicated in the table below.

Ethanol and tertiary butyl alcohol (TBA) for dehydration

TBA (ml)	TBA – Ethanol – water mixture		
	100 % ethanol (ml)	Distilled water (ml)	Duration
20	35	45	12
35	35	30	12
55	30	15	12
75	25	-	12
100	-	-	8
100	-	-	8

After dehydration, the materials were given two changes with TBA each at eight hours interval. During the final change, a pinch of cosin was added to stain the specimen super finally so as to locate them inside the wax embedding block.

# Infiltration and embedding

The dehydrated materials were transferred to a solution of liquid paraffin and TBA in 1:1 ratio for 1 h, Paraffin wax (boiling point 54 to 56 248C) was used for infiltration and embedding. The dehydrated materials along with TBA and liquid paraffin were transferred to a beaker containing wax. The materials were placed in the oven at 60°C for 12 h. Subsequent changes were administered with fresh molten paraffin wax at six-hour interval until the traces of TBA and paraffin were removed. The whole process was completed in 24 h. and the materials were embedded in paraffin wax contained in a paper boat. After cooling, the edges of the paraffin block were trimmed to form a cube.

#### **Microtome sectioning**

Uniformly thin ribbons of 10-µm thickness were cut using a Leico rotary microtome machine (JUNG RM 2045) and the ribbons were stretched out by gentle heating (40°C). Seed coat was given longitudinal section in the flat side and cotyledon was sectioned in the rear side.

The ribbons were cut into appropriate size and placed on the slide coated with a drop of 'Haupts' adhesive. These were flooded with 4 per cent formalin solution. The slide was slightly warmed until the paraffin stretched to lie flat on the slide. Excess fluid was drained off and the slides were shade dried for 10 to 12 h in a dust free chamber.

# **Staining**

Dried slides with ribbons were passed through Xylene – ethanol series for dissolving the wax and hydrating the sections for staining. The sequence of solvent treatment and the period are given below.

Steps	Minutes	
Xylene (I)	30	
Xylene (II)	30	
Xylene: Ethanol (1:1)	30	
100 per cent ethanol	10	
95 per cent ethanol	10	
70 per cent ethanol	10	
50 per cent ethanol	10	
30 per cent ethanol	10	
10 per cent ethanol	10	
Distilled water	10	
4 per cent ferrous ammonium sulphate	30	
1 per cent Erlich's hematoxylin	30	
Washing in running tap water	10	
The sections were stained with safranin stain		

# **Dehydration and mounting**

After staining the sections were dehydrated in ethanol – xylene series as given below.

Steps	Minutes
10 per cent ethanol	5
50 per cent ethanol	5
70 per cent ethanol	5
100 per cent ethanol	5
Ethanol: Xylene (1:1)	15
Xylene (I)	15
Xylene (II)	15

The sections were mounted with DPX mountant and observed under light microscope. Then, the photographs of the section of all stages were taken with 40X magnifications for embryo and 10X for seed coat

# 3.2.2.28. Measuring seed and embryo descriptors using Image Analysis System

Seeds as well as their respective decoated embryo of different developmental stages stating from 20 DAA to 80 DAA in rainy and 20 to 70DAA from summer crop were used for measuring shape descriptors. Freshly extracted seeds of CO 2 and Kerala local raised from both seasons were utilised for this experiment. Three replications of each ten seed / embryo were measured for every developmental stage. The instrument used, methodology followed and descriptors measured are described below.

# **Image analysis**

Image analysis was carried out using a Delta –T© (Delta–Insintrument Device – Cambridge, UK) image analyzer (Plate 3a) by running custom written software 'Win DIAS'.

For every replication ten seeds along with equal numbers of embryo were placed in a lighting box in such a way that embryo axes of seed, facing analyzer (Plate 3.) and longitudinal axes of the seed running parallel to the surface of the camera lens. Seeds were viewed with Video camera (DSP Surveillance colour charged- coupled device (CCD) camera CV-S3200/3300) using transmitted light, so that a binary image of the silhouette of the seed / embryo was recorded by the WinDIAS.

Images of the seed/embryo were graded by frame grabber to digitize the analogue image and stored in RAM of the computer. The image of the support was removed by software after image grabbing, which thus leaving an image of the objects consist four rows and five column of samples for geometric data measurements.

#### Data measurement

Before going for actual measurement, calibration was done by placing transparent plastic ruler on the light box illuminated from below. Ruler was aligned diagonally across the camera view and image was sharpened by adjusting the focus. Again, aperture adjustment was done until optimum colour and contrast was achieved. Input length was given in centimeter.

To measure the descriptors, from the menu tool, area meter was selected. After setting, the image was grabbed using image grabber and colour thresholds was given until the entire area was highlighted (Plate 3b). By logging the data, click in the measurement option in the WinDIAS, all the descriptor values were extracted by every time clicking individual objects. Values were checked from the review and mean data for the each parameter was summed up for average value in the WinDIAS itself. The images were saved in the Adobe ImageReady 7.0 version as a TIFF file format and their values were saved in Microsoft excel in the file name, My document/ash gourd descriptor. The details of measurements made (Figure 1) are as follows.

- i) Area
- ii) Perimeter
- iii) Length
- iv) Width

# v) Circularity

Circularity is square root of the ratio of the actual area of the object to the area of a circle with the same circumscribed diameter.

$$C = \begin{array}{|c|c|} \hline A \\ \hline A_D \\ \hline \end{array}$$

Where, A is the actual area of the object and  $A_D$  is the area of a circle with a diameter equal to the circumscribed diameter or length of the object.

# vi) Elongation

Elongation is the ratio of the length and width of an object.

# vii) Shape factor

Shape factor is the ratio of the actual perimeter to that of a circle with the same area.

$$S = \begin{array}{c} P \\ ---- \\ P_C \end{array}$$

Where, P is the perimeter of the object and  $P_C$  is the perimeter of a circle with the same area as the object.  $P_C$  is calculated as follows

$$P_C = 2 (\Pi * A)^{0.5}$$

Where, A is the actual area of the object.

# 3.2.2.29. Gel electrophoresis of total protein

The study material consist of CO 2 and Kerala local raised during summer and rainy seasons by discarding the immature seeds obtained from 15 DAA as they posses no appreciable size of cotyledon development. Stages viz., 20, 30, 40, 50, 60 and 70 DAA of summer season were subjected to electrophorosis based on total soluble protein. The same experiment was repeated for seeds harvested from rainy season by adding one more stage of 80 DAA.

# Methodology for electrophorosis

# **Defatting**

As a preliminary step samples were subjected to defatting since seed contain appreciable quantity of oil. Decoated seed materials were ground to a fine powder and samples taken in an eppendorf tubes and approximate quantity of defatting solution (Appendix IIa) added to the samples. To ensure settlement of samples with solution, they were kept in an oven maintained at 50°C for 3 hours. Before keeping oven

samples were votex for 2 minutes. Then, supernatants were decanted and again deffatting solution was added. These process was repeated for several times, until the oil content completely removed. After decanting defeated solution completely, the samples were dried. Ice-cold acetone was added to the dried powder and after 1 min. vortex, refrigerated for three hours. Then, samples were twice centrifuged at 8000 ppm for 30 minutes each. Again samples were allowed to dry.

#### **Extraction**

After defatting, the dried samples were added with 160 µl of Tris-HCl and NaCl (Appendix IIb) extraction buffer and allowed for overnight at room temperature. After centrifugation at 14,000 rpm for 30 minutes, the supernatant solutions were transferred to a fresh eppendorf. To this supernatant 600 µl of ice cold water was added so as get white turbid precipitation. All the tupes were fridge for 24 hrs and again centrifuged at 14,000 rpm for 30 minutes at 0°C. Supernatant was removed leaving remains of small pellet at the end of the eppenddorf. After drying 50 µl of Tris – Glycine extraction buffer (Appendix IIc) was added. To achieve complete disintegration of pellets, proper vortex was given.

After achieving complete disintegration of pellets, samples were added with 50 µl of working protein extraction buffer (Appendix IId) and allowed for overnight.

# **Running SDS-PAGE electrophoresis**

For electrophoresis, soluble protein were dissolved in SDS-Sample buffer as per the procedure (Hames, 1981) detailed above with required modifications for defatting.

Fifty  $\mu$  g of total protein was loaded on to 15% SDS gels. Electrophoresis was carried out at a constant current of 25 mA for about 6 hours at 20°C. Protein bands were visualized by Coomassie brilliant blue R250 staining. Gels were photographed by placing them in light box for examination and interpretation.

Dissolve 0.756 g of Tris in 50 ml of distilled water and adjust the pH to 6.8 using HCl and make up the volume to 100 ml.

# 3.2.3. Influence of fruit and seed position on seed quality

This study was conducted in CO 2 bulk crop raised during July, 2002 in rainy season.

Fifteen pistillate flowers for each fruit to be harvested were tagged on the nodes in the bottom (27-30<sup>th</sup> node), middle (37-40<sup>th</sup> node) and top (46 and above node) positions of the mother plants (Figure 2). The node numbers were fixed based on flowering pattern reported by Kamalanathan *et al.* (1972). Root bearing nodes that were completely attached with soil was not taken into account for all the treated plants. Fruits having different stages viz., 30,40,50, 60 and 70 days after anthesis were harvested for each fruit position. For every fruit stage, four fruits were taken for investigation with two replication, consisting two each. After harvest, the seed bearing part (seed cavity) was further dissected into three segments of equal length namely peduncular, intermediate and stylar segments (Plate 4).

The details of fruit position and seed position were presented below.

# **Fruit position**

Node number	Fruit position in the vine	Abbreviation
27-30	Bottom	В
37-40	Middle	M
>47	Тор	T

# **Seed position**

Fruit segment	Abbreviation
Peduncular	P
Intermediate	I
Stylar	S

Seeds together with mesocarp were removed and washed repeatedly in running tap water. After removing adhered mucilage completely, seeds were dried at room temperature for three days to get ten per cent moisture content. Afterwards, the treated seeds from different fruit and seed positions, were subjected to the following observations.

- **3.2.3.1. Seed moisture content** as detailed in 3.2.2.10
- 3.2.3.2. Dry weight of seed as detailed in 3.2.2.9
- **3.2.3.3. Germination** as detailed in 3.2.2.16

# 3.2.3.4. Field emergence

Field emergence test was conducted in a raised bed and seeds were sown in lines with a spacing of 5 x 10cm seed to seed and row to row spacing. Hundred seeds each with four replications were used per developmental stage. Copious watering was done with rose cane before and after sowing and subsequently whenever required. Per centage of seedling emergence was calculated at fourteen days after sowing.

# **3.2.3.5. Dry weight seedlings** as detailed in 3.2.2.19

# 3.2.4. Effect of fruit storage on seed quality

This study was conducted to find out the effect of post harvest storage of fruits on seed quality with respect to matured fruits (70 DAA). The well-matured fruits of CO 2 and kerala local harvested from the crop raised during summer season were stored in the storage room of Department of Seed Science and Technology. The fruits were kept in ventilated wooden racks in a scattered manner to expose them to ambient storage condition with an average temperature of 27°C and 70 % of RH.

The fruits were stored for 12 months. Seeds were extracted manually cleaned and dried and evaluated for following seed quality parameters.

# 3.2.4.1. Fruit weight loss

Every month, fruit weight was weighed individually and loss was expressed in Kilogram as well as per cent to know the relative weight reduction after the end of storage period.

- 3.2.4.2. Hundred seed weight as detailed in 3.2.2.11
- **3.2.4.3. Seed moisture content** as detailed in 3.2.2.10
- **3.2.4.4. Fresh seed weight** as detailed in 3.2.2.8
- **3.2.4.5. Dry seed weight** as detailed in 3.2.2.9
- **3.2.4.6. Speed of germination** as detailed in 3.2.2.15
- **3.2.4.7. Germination** as detailed in 3.2.2.16

- **3.2.4.8. Shoot length** as detailed in 3.2.2.17
- **3.2.4.9. Root length** as detailed in 3.2.2.18
- **3.2.4.10. Dry weight of seedling** as detailed in 3.2.2.19
- **3.2.4.11. Vigour index** as detailed in 3.2.2.20

# 3.2.5. Standardisation of a suitable method of seed extraction, separation cleaning, washing and drying

The matured harvested fruits (70DAA) from the bulk crop cv. CO 2 raised during rainy season, were formed the materials for this experiment. The harvested fruits were stored in the laboratory to carry out fermentation, washing and drying experiments which was done for five months with monthly interval, using nine fruits per month with three replications of three fruits each.

#### **Extraction methods**

Generally ash gourd fruits are cut longitudinally and seeds along with mucilaginous substances removed by scooping from the seed cavity which is laborious and requires a lot of time (Plate 5a). In order to reduce the time, fruits are cut into four or five slices depending upon the size of fruit and the entire seed cavity was removed like a disc and the content was subjected to fermentation process (Plate 5b).

The seeds along with pulp and juice were collected in a plastic container and divided into three equal quantities. Accordingly, all the fruits were extracted, divided and subjected to two different fermentation methods namely (i.) sealed fermentation in closed plastic bag for 72 h at 25°C in the dark and (ii.) open fermentation in plastic bucket for 72 h at 25°C in the room condition. Seed extraction without fermentation formed the control.

For sealed fermentation 300 gauge thickness polyethylene bags were used and after filling the seed content, the bags were sealed. The seeds obtained from all the three methods were subjected to cleaning washing and drying as described below.

# Seed cleaning, washing and drying methods

- $T_1$  Manual separation followed by sun drying for 12h.
- $T_2$  Manual separation and drying for 72 h at room temperature at 25  $\pm$  5°C followed by sun drying for 12h
- $T_3$  Washing for 15 min. in running tap water followed by sun drying for 12 h.
- $T_4$  Washing for 15 minutes and drying for 72 h at room temperature at 25  $\pm$  5°C followed by sun drying for12 h

The seed samples taken from the above 12 treatments (3 extraction methods and 4 separation and drying processes) were subjected to the seed quality evaluation.

- **3.2.5.1. Speed of germination** as detailed in 3.2.2.15
- **3.2.5.2. Germination** as detailed in 3.2.2.16
- **3.2.5.3. Shoot length** as detailed in 3.2.2.17
- **3.2.5.4. Root length** as detailed in 3.2.2.18
- **3.2.5.5.** Dry weight seedling as detailed in 3.2.2.19
- **3.2.5.6. Vigour index** as detailed in 3.2.2.20

# 3.2.6. On Farm Trial for test verification of the best method of seed extraction and drying

After assessing the best method of seed extraction and drying, its effectiveness, usefulness, acceptability and adaptability were test verified at the seed producers unit for large-scale adaptation of sealed fermentation, a successful and best method of seed extraction, where, instead of polyethylene bag, plastic drum with airtight lid was used for closed fermentation. The address of the seed producer is furnished below.

Mr. K.R. Rangaraj S/O. Ranganathan Kondamuthunur Sathyamangalam (Taluk) Aryappampalayam (Post) Coimbatore (Dt.)

Phone: 04285 251382

This study was conducted with 45 MT ash gourd variety CO2 fruits harvested from two acres.

The economics of sealed fermentation, washing and drying methods for 45 MT of fruits, harvested from two acres of land were worked out. The appreciation letter given by the seed producer is also furnished.

# 3.2.7. Statistical analysis

The data collected from various experiments were analysed statistically, adopting the procedure described by Panse and Sukhatme (1999). Wherever, necessary, the per cent values were transformed to angular (Arc sine) values before analysis. The critical difference was worked out for five per cent probability.

# CHAPTER IV RESULTS

Results from the experiments carried out with ash gourd varieties CO 2 and Kerala local on flowering behaviour, fruit and seed development and maturation as influenced by varieties and seasons, influence of fruit and seed position on seed quality, effect of fruit storage on seed quality, standardisation of a suitable method of seed extraction, separation, washing and drying and On Farm Trail on seed extraction are presented in this chapter.

# 4.1. FLOWERING BEHAVIOUR AS INFLUENCED BY VARIETIES AND SEASONS

# **4.1.1.** Days to male and female flower bud initiation to anthesis (Table 1)

There was no much difference was observed among male and female flowers for days from bud initiation to anthesis. No significant results were obtained among variety and the interactions between season and varieties. However, there was a significant difference due to the season. During summer, male and female buds took 4.4 to 5.7 days from visible initiation to anthesis, whereas during rainy, it was reduced to 2.8 to 3.1 days.

# 4.1.2. Days to first male and female flower initiation (Table 1)

Crop sown in the rainy season (July-December) had an early emergence of male and female flowers in both cultivars. The male flowers were emerged 64 days in summer and 48.8 days in rainy, whereas female flowers took 66 and 54 days during respective seasons. The variety Kerala local had an early male and female flower initiation of 54 and 59 days respectively, whereas CO 2 took 58 and 60 days for these phases, respectively.

# 4.1.3. Node at which first male and female flower appeared (Table 1)

Female and male flowers appeared early in rainy season in both varieties. The variety CO 2 had earliness in male and female flower appearance at 12<sup>th</sup> and 16<sup>th</sup>

nodes respectively, whereas in Kerala local, the flower appearance was noticed in 15 and 19<sup>th</sup> node, respectively. In summer, male and female appeared in 17 and 25<sup>th</sup> node in CO 2 and these phases appeared in 16 and 26 nodes in Kerala local.

# 4.1.4. Sex ratio and number of fruits per vine (Table 1)

Crop raised in summer had wider sex ratio of 21:1 and it was narrowed down to 17:1 in rainy crop. Among varieties, Kerala local had wider sex ratio in summer (22:1) than CO 2 (20:1). During rainy season both varieties had same (17:1) sex ratio.

Kerala local had as many as 5.8 fruits per vine in rainy and two numbers in summer. This was closely followed by CO 2 (4.4 and 1.4number of fruits per vine, respectively) with an average numbers of 2.9 which was lower than Kerala local (3.8)

# 4.2. FRUIT AND SEED DEVELOPMENT AND MATURATION AS INFLUENCED BY VARIETIES AND SEASONS

# 4.2.1. Fruit weight (Table 2)

The weight of developing fruits had increased gradually from the day of anthesis and till its penultimate maturity stage in rainy season and slightly earlier in summer. The mean weight of fruits increased from the initial weight of 178g (5 DAA) to 6009 g at 60 DAA in summer and 7753 g (70 DAA) during rainy season. Computed mean fruit weight revealed that maximum fruit weight attained in the growth stages *viz.*, 55 (6408 g), 60 (6612 g), 65 (6549 g) and 70 DAA (6623 g). Both varieties had reached their peak fruit weight in 70 DAA. At this stage the highest weight (7621 g) was observed in CO 2. The Kerala local had comparatively less fruit weight (5625 g). The variety CO 2 had its maximum weight (6812) at 60 DAA and it was extended (9104 g) upto 70 DAA during rainy. The Kerala local could reach maximum weight (5308 g) from 55 DAA in summer and took 70 DAA to attain maximum weight (6402 g) in rainy. Large variations were observed for fruit weight by all the factors. Growth stages had exerted maximum effect followed by variety and season.

From this, it is evident that, in rainy season, fruit weight increase was proportionate and gradual, and it extended upto 70 DAA and at this stage the mean fruit weight was 7753 g against 189 g recorded at 5 DAA. Whereas in summer, maximum weight was attained (6009 g) in 60 DAA itself and thereafter, it declined. CO 2 and Kerala local could produce fruits with maximum weight of 9000 g and 6402 g at 70 DAA in rainy, whereas in summer the weight was 6812 and 5308 g in early stages of 60 and 55 DAA, respectively.

# **4.2.2. Fruit circumference (Table 3)**

The overall mean data indicated that the circumference of fruits increased from 19.4 cm at 5 DAA to 73.7 cm at 70 DAA. In both the rainy and summer season, maximum values (74.4 and 72.9 respectively) were observed at 70 DAA. The variety CO 2 could reach as high as 87.8cm in rainy, and 85.9 cm in summer, whereas comparatively lesser values (62.1 and 57.0) were recorded in Kerala local for the respective season. In summer, maximum value could be obtained in the earlier stage (60 DAA) itself. The variety mean indicated that CO 2 and Kerala local attained their maximum circumference of 87.8 and 60.5 cm at 70 DAA and thereafter, they showed a declining trend. Both varieties had performed fairly well during rainy season for fruit circumference. Here again, developmental variation superceded the other factors for the significant difference.

#### 4.2.3. Fruit length (Table 4)

There was a gradual increase in fruit length from the initial stage of 50 DAA (12.8 cm) to last maturity stage of 70 DAA (38.8 cm) in rainy season. The increase in length was extended up to 80 DAA in rainy, whereas in summer, maximum length was recorded in 60 DAA itself. In the subsequent stages, a slight decrease was noticed in summer. The variety CO 2 took 60 DAA to attain 43.0 cm length, whereas Kerala local attained 35.9 cm in 65 DAA.

#### 4.2.4. Fruit diameter (Table 5)

The maximum fruit diameter (22.0 cm) was achieved at 55 DAA. In rainy season, marginal increase in diameter was noticed upto 70 DAA. Whereas in summer, maximum values were recorded at 55 DAA itself and however the values were very low (20.7 cm) compared to rainy season (23.2 cm). Variety CO 2 had expanded its diameter to 26.6 cm at 60 DAA, whereas Kerala local could reach 19.3 cm only, for the corresponding stage (55 DAA).

# 4.2.5. Pulp thickness (Table 6)

Fruits of ash gourd had continuous growth of pulp from 5 DAA to 65 DAA, recording a pulp thickness of 3.6cm and 18.4 cm, respectively. Beyond this stage (65 DAA), the pulp content was static with slight decline trend. During the rainy, appreciable amount of addition of pulp was noticed upto 60 DAA and had decreased in subsequent stages. Summer fruit also reached its maximum value (10.8 cm) at 60 DAA. As per the variety mean, Kerala local had showed a meager thickness of pulp (7.7 cm) even after attaining full maturity, whereas CO 2 had double the amount of thickness (16.5 cm) at 60 DAA.

## **4.2.6** Seed cavity diameter (Table 7)

Completely different trend of results was observed with regard to seed cavity diameter. Kerala local had thicker seed cavity than CO 2. This was one of characteristic difference noticed between these two varieties. Overall mean diameter had increased from 1.3 cm (5 DAA) to 10.2 (55 DAA) and declined slightly thereafter. Maximum value of 10.4 cm was observed during rainy season at 60 DAA and during summer at 55 DAA itself. Varietal mean indicated that Kerala local had 11.9 cm diameter at 60 DAA and CO 2 realised its maximum value of 8.8 cm five days earlier i.e., 55 DAA itself. Unlike other characters, a difference due to season was comparatively less for seed cavity diameter. In summer, CO 2 had achieved it peak value (8.9 cm), as early as 45 DAA and beyond this stage there was no much

change in values. In rainy season, CO 2 took 55 DAA to reach 8.9 cm seed cavity diameter and afterwards a slight decline was noticed. Kerala local also had followed the same trend of growth during both season, but their values were much higher. Kerala local had recorded as high as 11.8 and 11.9 cm diameter in summer and rainy season, respectively whereas during the same period CO 2 could reach 7.9 and 8.4 cm only.

# 4.2.7. Number of well-filled good seeds (Table 8)

As growth and development proceeded, there was a significant improvement in good seed number. As per the overall mean, appreciable amount of filled good seeds could be recovered from the stage 20 DAA onwards, by recording 252 numbers, which increased to 1655 at 50 DAA, and thereafter it maintained. The rainy crop had comparatively lesser (1747) number of seeds even after attaining peak maturity (70 DAA). At the same stage, summer crop had as high as 1863 seeds. Kerala local had a large number of seeds, which initially began with 300 and 431 in summer and rainy respectively, and reached as high as 2805 and 2503 at the end of maturity (70 DAA). The variety CO 2 had small number of well filled good seeds in seed cavity, by recording 993 in summer and 991 in rainy at 65 and 70 DAA respectively.

# 4.2.8. Fresh weight of seed (Table 9)

Fresh weight of seed had a significant difference due to all the factors and their interactions. The initial fresh weight of seeds (54.1 g) had steady progress as growth stages advanced and reached its maximum weight (115 g) at 60 DAA. In rainy season, maximum weight of 137.3 and 137.2 g was obtained at 65 and 75 DAA respectively. Though summer crop had shown lower fresh weight, their peak weight occurred early (55 DAA) when compared to rainy season. The variety CO 2 had recorded an initial weight of 38.7 g and picked up during the subsequent stages and reached maximum weight (87.5 g) at 60 DAA. Beyond this stage, there was a slight decrease in weight. The initial value recorded at 20 DAA itself was an evident of

superiority of rainy season over summer for fresh weight. Both Kerala local (83.1 g) and CO 2 (42.2 g) had higher values in rainy season when compared to (56.1 and 35.2 g) summer crop at 20 DAA. The highest weight of 188 and 103 g was recorded in Kerala local during rainy and summer seasons, respectively whereas CO 2 had 192.6 and 82.3 g during the respective season at growth stages between 55-60 DAA.

# **4.2.9.** Dry weight of the seed (Table 10)

The dry weight of seeds per fruit, which was 26.2 g on 20 DAA, had increased significantly and registered a maximum value of 63.9 g in 55 DAA. The fruits produced in rainy season had 35.2 and 73.7 g of dry weight of seed during initial and peak growth stages, respectively. Summer crop had lower dry weight of 17.1 and 54.2 g during the respective stages. The variety Kerala local had reached as high as 87.6 g in 60 DAA and CO 2 showed very low weight of 38 g per fruit as per individual variety mean. Both varieties had showed a sharp decline in weight of dry seeds beyond 60 DAA in both seasons.

#### **4.2.10.** Moisture content of seed (Table 11)

Seed moisture declined progressively with time from anthesis until penultimate final maturity of fruit development. According to overall mean, initial seed moisture content (64.8 per cent) registered in 20 DAA declined to 49.9 per cent during ultimate developmental stage of 70 DAA. During rainy season, the initial and peak moisture content recorded were 70.9 and 50.2, whereas summer recorded 58.7 and 49.6 per cent during the respective stages. The variety Kerala local showed a drastic reduction from the initial value of 66.2 to 42.1 per cent and such drastic reduction was not observed in CO 2; instead it had steady decline from 63.3 to 57.6 per cent. As far as season is concerned, during rainy season, seeds from both varieties maintained higher moisture content in all the stages of development which were 66.1 and 57.0 per cent in CO 2 and 75.7 and 43.4 per cent in Kerala local at

initial and final maturity, respectively. Whereas, during summer season lower level of seed moisture was recorded in all the stages of development, irrespective of variety.

#### 4.2.11. Hundred seed weight (Table 12)

As per overall mean, 70 DAA had maximum (7.14 g) hundred seed weight and it was 7.93 g in rainy season and 6.34 g in summer. During the early stage of development (20 DAA) itself, Kerala local registered a higher weight of 3.24 g and it was lower (2.21 g) in CO 2. Both varieties had gradual increase in weight as seed growth and development progress and attained slight weight increase after 50 DAA. Kerala local reached a peak value of 9.97 g during rainy season and 6.83 g during summer. The variety CO 2 also had same trend of weight increase and attained maximum (6.14 g) during rainy season at 70 DAA and 5.89 g during summer at 65 DAA.

#### **4.2.1.2.** Fruit to seed recovery (Table 13)

With respect to overall mean, recovery of well-filled good seeds was the highest (1.06 per cent) in 55 DAA and beyond this stage it had slight decline. Fruits produced in rainy had the maximum recovery of 1.18 per cent (55 DAA) and lower recovery (0.96) was realized in summer even after reaching matured stage of 65 DAA. Good seed recovery was reasonably high during the late stages of seed development in both seasons. The mean recovery recorded in CO 2 was 0.51 per cent and during summer it was 0.48 per cent and in rainy season crop the recovery was 0.55 per cent during the stages of 60 to 70 DAA. The same trend of result was obtained in Kerala local and it registered an average of 1.85 per cent at 55 DAA and maintained its healthier lead in all the matured stages *viz.*, 60-80 DAA. The recovery was 1.8 per cent in rainy and 1.4 per cent in summer. However, there was no much difference between two seasons as per the average recovery recorded by both varieties. As far as CO 2 was concerned, maximum seed recovery of 0.50 and

0.55 per cent was registered during 55<sup>th</sup> DAA. During the same period of maturity stage, Kerala local had as high as 1.35 and 1.85 per cent of seed recovery.

# 4.2.13. Cotyledon thickness (Table 14)

According to overall mean, embryo thickness undergone gradual increase from 20 DAA onwards. Initially (20 DAA) it had 0.16 mm and reached maximum (1.97 mm) during 70 DAA. In rainy season, it was 2.62. 2.05 and 2.08 cm at 70, 75 and 80 DAA respectively and 1.88, 1.87 and 1.92 mm during summer season for the respective growth stages. The variety Kerala local had slightly higher thickness (2.06 mm) at peak maturity stage (70 DAA) than CO 2 (1.88 mm), as per the individual varietal mean values. However, it had only slight differences between summer and rainy season in all the growth stages and variety. But during rainy season, slight increase of values was observed beyond 70 DAA in both varieties even after assuming satisfactory growth in their early stages. The cotyledanary growth was 1.96 and 1.98 mm (CO 2) and 2.13 and 2.18 (Kerala local) during these extended period of seed maturity (75 and 80 DAA). Summer crop had maximum embryo thickness on 70 DAA and it was 1.83 mm in CO 2 and 2.01 mm in Kerala local.

#### 4.2.14. Cotyledon weight (Table 15)

According to pooled mean, like cotyledon thickness, embryo weight also had a gradual increase from the starting stage of 20 DAA onwards. The increase in weight was continuous as the growth stages progress and from the initial value of 0.016 mg it had reached its peak 0.084 mg at 75 DAA and maintained in subsequent stages.

The variety Kerala local found to have slight increase in embryo weight over its counterpart even beyond 70 DAA. They recorded 0.185 and 0.186 mg in 75 and 80 DAA, respectively during rainy season, whereas during summer it was restricted to 0.173 mg. The variety CO 2 also had a slight increase recording 0.153 and 0.154 mg

during rainy season and restricted to 0.152 mg during summer season during the respective growth stages as in Kerala local.

#### 4.2.15. Speed of germination (Table 16)

Speed of germination was very less (0.17) initially (30 DAA) and reached the highest (13.3) at 65 DAA. Immature seeds in the middle stages of development had lower speed of germination recording 2.3, 5.6. 9.4, 10.9 and 12.1 at 35, 40, 45, 50, and 55 DAA respectively and reached a maximum level of 13.2 at 60 DAA. Though, rainy season seed failed to germinate initially (30 DAA), but reached the highest speed (13.8) at 70 DAA and remained the same upto 80 DAA. Summer crop had an early (30 DAA) response. After 60 DAA, speed was declined to 13.5 and 12.5 at 65 and 70 DAA, respectively.

#### **4.2.16. Germination (Table 17)**

The developing seed started to germinate (1.6 per cent) at 30 DAA and peak value (81 per cent) was registered in 65 DAA and then it got decreased (79.4 per cent) slightly at 70 DAA. In rainy crop, the highest germination of 84.5 per cent was recorded at peak maturity (80 DAA). The summer crop had responded early (65 DAA) by registering high germination (83.0per cent) and further it got declined to 76.5 per cent at 70 DAA. The performance of CO 2 and Kerala local, the former had a germination of 2 per cent at early stages and reached maximum of 85 per cent at final stage which was higher than Kerala local (1.25 and 76.5 per cent, respectively).

In summer season, early germination was recorded in both CO 2 and Kerala local recording 4.0 and 2.5 per cent. The seeds of both the varieties did not germinate at 30 DAA in rainy crop. As growth and development progress, increase of germination was gradual and CO 2 reached its peak value of 86.5 and 82.5 per cent at 70 DAA during rainy and summer season, respectively. Summer crop of Kerala local

had early peak germination of 80.5 per cent at 65 DAA and took 80 DAA to reach 84 per cent germination in rainy season.

#### **4.2.17. Shoot length (Table 18)**

The seedlings from 70 DAA had maximum (16.8) shoot length as per average mean of varieties and seasons. Seeds extracted from initial stage (30 and 35 DAA) had shorter shoot length (4.5 and 6.8 cm). As the growth progressed shoot length increased gradually to 6.8, 12.7, 13.4, 14.8 and 15.3 cm during the stages of 40, 45, 50, 55 and 60 DAA respectively. The longest shoot was recorded at 60 DAA and showed a decline in the subsequent stages (65 and 70 DAA). Rainy crop had the highest shoot value of 18.3 cm at 75 DAA and the highest value was achieved in summer crop at 60 DAA itself; beyond this stage, a slight decline was noticed. The varieties CO 2 and Kerala local had peak values of 16.4 and 17.9 cm during summer at 60 DAA and 18.4 cm (75 and 80 DAA) during rainy season, respectively. During summer season, CO 2 had an early germination and resultant seedling had 4.1 cm length, which was higher than its counter part Kerala local (1.8 cm) at initial stage (30 DAA).

#### **4.2.18. Root length (Table 19)**

Seeds from initial stages (30 DAA) had less (4.2, 9.4) root growth and gradually increased and reached 13.4, 16.0, 16.3 and 17.4 cm at 40, 45, 50 and 55 DAA, respectively. The maximum root length (18.4) was recorded at 60 DAA and showed a slight reduction further during 65 and 70 DAA. Rainy crop had slow progress initially, and recorded zero and 7.9 cm of root length (30 and 35 DAA, respectively) and reached 18.5 cm at 60 DAA and maintained its growth (18.4 cm) upto 80 DAA. Summer crop had an early germination with satisfactory root growth (8.4 cm) and continued upto 60 DAA and during the course of development it had the root growth of 10.9, 12.7, 15.4, 15.6 and 17.1 cm during 35, 40, 45, 50, 55 DAA. After 60 DAA, summer crop had comparatively more reduction (18.1 and 17.5 cm)

during 65 and 70 DAA. The variety CO 2 had 12.6 cm root growth in summer season at 30 DAA, whereas in rainy season it registered zero value. Likewise, Kerala local recorded 4.3 cm in summer and zero value in rainy season at 30 DAA.

# 4.2.19. Dry weight of seedling (Table 20)

Seedlings from initial stages (30 and 35 DAA) had less (4.8, 15.7 mg) dry weights and seedling weight increased to 28.9, 32.3, 33.5 and 34.6 mg during 40, 45, 50 and 55 DAA. The maximum seedling weight (37.0 mg) was recorded at 60 DAA and had a slight decline further during 70 and 75 DAA. Seedlings produced from rainy crop showed the highest seedling weight of 41.9 mg at peak maturity (80 DAA), whereas summer crop had early increase in seedling weight (37.6 mg) during 60 DAA. Corresponding growth stage of 60 DAA in rainy crop also had higher weight (40.5 mg). The both CO 2 and Kerala local showed continuous accumulation of seedling dry weight of 39.9 and 38.2 mg upto 60 DAA, respectively.

#### **4.2.20. Vigour Index (Table 21)**

As per the overall mean, 65 DAA had maximum (2833) vigour index. During rainy season, the vigour index was maximum (3081) at 80 DAA, whereas in summer, it was maximum (2872) at 60 DAA.

#### **4.2.21.** Viability evaluation by tetrazolim test (Table 22)

The trend of TZ viability test observed at different stages of seeds was closely followed the results that were obtained in germination test. Though the intact cotyledon with differentiated embryo was formed at 30 DAA, they failed to stain in rainy crop, whereas summer seed exhibited a ten per cent staining in the whole cotyledon as well as embryonic axis. Seeds from early stages (30 DAA) had lowest (5.0 per cent) viability and higher viability was (84.6 per cent) recorded at 65 DAA (Plate 6). The staining of cotyledon indicated that the variety CO2 responded well and

registered cent per cent viability after attaining the maturity of 70 DAA and remained the same during the subsequent stages in rainy season. Kerala local had 94.5 per cent during the peak maturity (70 DAA). During summer, there was no much difference in all the stages between two varieties.

#### **4.2.22.** Oil content (Table 23)

The increase in accumulation of oil/seed was gradual as growth stages advance, only numerical increase in values was observed and they are not significant to each other.

#### 4.2.23. Dehydrogenase enzyme activity (Table 24)

The increase in dehydrogenase activity was very much visible as growth progress from 40 DAA to 80 DAA, and the initial growth stage of 30 DAA had registered zero activity. Numerical increase in values was noticed in all the season, in both varieties as growth and maturity progress. However, the difference was not significant due to variety, season and their interactions.

#### 4.2.24. Protein content (Table 25)

Protein accumulation continued upto 50 DAA and a marginal increase were noticed during the subsequent stages of seed development. At 30 DAA itself, substantial amount of protein content (31.0 per cent) had accumulated and peak accumulation (37.8 per cent) was recorded at 60 DAA. Seeds from rainy crop had steadily accumulated the protein and more per cent (38.0 per cent) was achieved at 75 DAA. Whereas summer crop had accumulated more or less same per cent (37.5 per cent) as that of rainy season at 70 DAA.

# 4.2.25. Descriptive characteristics of fruit

# a. Summer season

Days After Anthesis	Descriptive characteristics of fruits
5-10	Light green fruits with scattered white spot. dark green at pedicel end, persistent corolla, highly pubescent
10-15	Prominent white spots mostly at stylar end in fruit; fruits are light green in colour, pedicel dark green, ashy coat at pedicel end, pubescent.
15-20	Ashy coating covered half portion of rind, intensity of pubescent decreases, pedicel thickened.
20-25	Ashy coat covers entire fruit, but with less intensity except ground portion, pubescent
25-30	Ashy coat covers entire rind surfaces and become thicker and sticky, pubescent reduced.
30-35	Rind of fruit became harder, pubescent at less intensity, dark green pedicel.
35-40	Ashy coat became very thick and sticky, prominent longitudinal groves formed.
40-50	Pubescent disappears, depression formed at stalk end indicating start of maturity, grooves become prominent; pedicel became light green, no longer preferred for vegetable purpose.
50-60	Pubescent disappeared completely, pedicel slightly dried, fruit rind very hard.  If we give protracted cut, crack forms in rind, pithy pulp, maximum peduncular depression
60-70	Summer crop senescence in the form of yellow leaves started, pedicel completely dried, ashes become powdery, total crop duration approximately 170 days

# b. Rainy season

Days After Anthesis	Descriptive characteristics of fruits
5-10	Light green fruits with scattered white spot, dark green at pedicel end, persistent corolla, highly pubescent
10-15	Early ash coating not observed as noticed in summer, which was delayed (Plate 7a)
15-20	Ashy coating begins here and remaining characters are in agreement with summer crop (Plate 7b)
20-25	Ashy coat covers entire fruit, but with less intensity except ground portion, pubescent
25-30	Ashy coat covers entire rind surfaces except few patches (Plate 7c)
30-35	Rind of fruit became harder, pubescent at less intensity, dark green pedicel, pubescent reduced.
35-40	Rinds become slightly hard, but not equivalent to summer, prominent longitudinal groves just developed
40-50	Ashy coat intensity increased, sticky and pulp is still looks tender (Plate 7d)
50-60	No pithiness as in summer crop, pedicel becomes light green, resistant felt while cutting the fruit pulp,if we give protracted cut, crack not formed in the rind as observed in summer,maximum peduncular shoulder depression
60-70	Angular, light green pedicel, depressed peduncular shoulder, thick ashy coat fruit longevity continued (Plate 7e)
70-80	Total crop duration continued beyond 180 days, fruits are still intact with vine but gets fungal attack in matured fruits (Plate 7f)

# 4.2.26. Descriptive characteristics of seed

# a. Summer season

Description	Days After Anthesis	Seed characteristics in summer
Pre-embryonic	5	Globular embryo, developing ovary
Pre-cotyledon	10	Heart shaped embryo, remnant of seed coat and water filled cotyledon and distinctly separate.
Early-cotyledon	15	Small papery cotyledon, seed coat develops, seed tightly packed in cavity, creamy seed, liquid cotyledon.
Enlargement I	20	Small embryo, cotyledon develops and thickens, developing seed coat, groves developed in margin of seed, seeds can be removed but attached through funicle
Enlargement II	25	Prominent grooves at seed margin, cotyledon thickened, seeds could be removed without much effort, slightly yellow seed coat.
Filling	30-35	Cotyledon thickening, seeds easily come out from placenta, they became harder (Plate 8a)
Filling &  Maturation I	40-50	Cotyledon tightly folded, air space noticed in seed cavity (Plate 8b)
Filling &  Maturation II	50-60	Seeds attain maximum width and thickness, fully developed with prominent margin (Plate 8c)
Maturation III	60-70	Light yellow and maximum size in summer, completely detached from placenta, desiccation started, seeds freely comes out (Plate 8d and 8e)

#### b. Rainy season

Days after Anthesis (DAA)	Seed characteristics
5-40	No marked difference observed between summer and rainy
40-50	No air space in seed cavity, still seeds attached with placenta
50-60	Seeds attain maximum width and thickness, fully developed with prominent margin.
60-70	No desiccation, but seeds detached from placenta
70-80	Air space visible, but still seeds are fresh and continued to grow, desiccation was delayed,

# 4.2.27. Histological studies

Structure and development were studied based on arbitrary fixation of stages as days after anthesis. The growth was continuous and hence, whatever structural changes noticed in the ovule during the stages studied *viz.* 5,10,15, 20, 30, 40, 50, 60, 70 and 80 were furnished in the result. The seed coat development was studied 20 DAA onwards.

# Structural changes in testa

Localized multiplicity nature of the outer integument was not visible distinctly in early development of seed coat (20 DAA). Early stages had mostly showed sclerenchymatous layer and testa structure was so compact that result in exerting resistant to embryo expansion and epidermal was visible without undergoing divisions. Sclerenchymatous layers were not at all demarcated from other hypodermal sclerotic layers. Demarcation and layers were clearly visible only during 30, 40 and 50 DAA but proportionately. In intermediate maturity (40, 50 and 60 DAA) enlargement of cells was noticed, which was proportionate as per the increase of

growth stages. Periclinal division of ovular hypodermis which was compressed by the formation and maximum growth of this layer, was seen and readied in matured seed for testa split into two parts.

Divisibility increased proportionately as light multiple small cells; thickening enlargement and clear enlargement of cells as layers in these intermediate stages as growth proceed. Subsequently, testa has become harder. In matured seeds (60 DAA) outer epidermis was clearly visible. Over-matured seeds were not amenable to microtome and hence, picture visualized as a mass of coalesced cells without any demarcation. In advanced matured seeds, all the epidermal, subepidermal and pigmented layer cells were crushed into non-structured cell walls stacked on the outer or inner face of the palisade layer.

In all the young stages (20, 30 and 40 DAA), the radial and basal walls of the palisade cells were heavily thickened to form a strong mechanical constraint and to protect the embryo. In contrast, matured testa was not thickened consistently. In adaxial side, some palisade cells were not thickened. These differences in structure testa may represent reductions in mature seed testa integrity. Moreover, in dry over-matured seeds, the testa became hard and resulted in difficulty in taking sections and here even un-thickened palisade cells were also crushed into non-cellular structures.

#### Structural changes in embryo

The globular shaped embryo was observed 5 days after anthesis. During 15 DAA, the embryo was heart-shaped with cotyledon ridges developing. At this stage, embryo differentiated into root and plumule. At 20 DAA, the entire cotyledon developed and thickened to accommodate embryo growth. At this stage, beginning of expansion of cells was noticed. At 30 DAA, embryo axis continued its elongation and cotyledon thickening continued until 70 DAA. During full maturity (70 and 80 DAA), axis had curvature in the centre of tip. Expansion of cells was

noticed and reached peak. Multiple growth was noticed in one section at peak maturity (80 DAA) with cells of maximum expansion. This probably indicates the over-maturity and prepared for immediate germination. In matured seeds starting from 60 DAA to 80 DAA, embryonic axis had attained its maximum size.

Sections of root structure revealed that during the early stages, cells were packed closely, and were heavily thickened without any expansion. In the next stage (30 DAA), beginning of expansion of cells was noticed mostly in the tip of the root cap. Expansion of cells from top to bottom was clearly visible in the next stage (40 DAA). Maximum expansion and disintegration of cells and root cap was noticed in 70 and 80 DAA coupled with attainment of matured size and growth.

## 4.2.28. Measurement of seed and embryo descriptors using image analysis system

The ash gourd seeds of CO 2 and Kerala local with different stages were measured by computer based image analysis system.

#### i. Area of embryo and seed (Table 26 and 27)

Area of the cotyledon showed significant differences due to season, variety and their interactions during growth and development of seed. The initial and peak area recorded were 0.27 and 0.35 cm<sup>2</sup> at 30 and 70 DAA, respectively. During rainy season, increase in values was steady and peak value (0.39 cm<sup>2</sup>) was registered at 80 DAA. On the other hand, cotyledons of different stages from summer season had early growth and its maximum area (0.39 cm<sup>2</sup>) coincided in 70 DAA. The variety Kerala local had reached its peak value (0.46 cm<sup>2</sup>) at 80 DAA during rainy season and 0.43 cm<sup>2</sup> during summer.

As far as seed is concerned, they attained steady growth and reached its maximum value (0.74 cm<sup>2</sup>) at peak maturity stage (70 DAA). During rainy season the area was extended (0.78 cm<sup>2</sup>) upto 80 DAA, whereas in summer season early peak (0.82 cm<sup>2</sup>) was registered in 70 DAA itself. The variety Kerala local had showed

promising growth in terms of area (0.79 cm<sup>2</sup>), which was higher than CO 2 (0.64 cm<sup>2</sup>) at 60 DAA. The performance was reflected in both season and Kerala local registered higher growth of 0.90 (70 DAA) and 0.94 cm<sup>2</sup> (80 DAA) during summer and rainy season respectively. During the same period, CO 2 showed lower value of 0.74 and 0.63 cm<sup>2</sup>, respectively.

# ii. Perimeter of embryo and seed (Table 28 and 29)

Cotyledon had the maximum perimeter (2.74cm) on 70 DAA as per overall mean and during rainy and summer seasons it extended to 2.64 and 2.63 cm respectively. Variety mean also had same trend of result throughout the developmental stages. The variety Kerala local had showed higher perimeter reading than CO 2 and former attained a peak of 2.92 cm (80 DAA) and 2.8 cm (70 DAA) and latter attained 2.36 cm (80 DAA) and 2.5 cm (70 DAA) during rainy and summer season, respectively.

Perimeter of seed was higher than cotyledon and it was maximum (3.47 cm) at 70 DAA. During rainy season, it was slightly extended to 3.63 cm (80 DAA) and during summer season it had 3.67 cm at 70 DAA itself. Kerala local had maximum perimeter recording 3.87 cm (70 DAA) and 4.06 cm (80 DAA) during summer and rainy seasons respectively. Whereas, it was 3.48 cm (70 DAA) and 3.21 cm and (80 DAA) in CO 2 respectively.

#### iii. Length of embryo and seed (Table 30 and 31)

Significant differences were observed for all the factors studied except variety and season interaction. Maximum length was attained in 60 DAA itself for cotyledon (0.93 cm) and it took 70 DAA for seed to attain maximum length (1.27 cm). During rainy season, both cotyledon and seed had maximum values of 1.05 and 1.29 cm on 80 DAA, whereas summer crop had early peak values (1.04 and 2.37 cm) in both cotyledon and seed respectively at 70 DAA. The Kerala local had lengthy cotyledon

(1.01 cm) and seed (1.34 cm) during peak maturity (70 DAA), when compared to CO 2 (0.91 and 1.22 cm).

#### iv. Width of embryo and seed (Table 32 and 33)

All the parameters observed were found to show a significant difference except variety for width of cotyledon and seed measured through image analysis system.

As per the overall mean, maximum width was obtained in 70 DAA in cotyledon (0.50 cm) and seed (0.76 cm). During rainy season, both cotyledon and seed reached their maximum width of 0.50 cm and 0.82 cm at 80 DAA, whereas in summer season they were 0.53 and 0.79 cm respectively. Extended growth was proved at 80 DAA as both CO 2 and Kerala local had maximum width of 0.53 and 0.53 cm for cotyledon and 0.76 and 0.82 cm for seed, respectively during rainy season. During summer, maximum value was achieved at 70 DAA. The width of cotyledon and seeds were 0.51 and 0.55 cm in the case of CO 2 and 0.74 and 0.83 cm in the case of Kerala local respectively.

#### v. Circularity of embryo and seed (Table 34 and 35)

Circularity had showed increasing trend in both cotyledon and seed irrespective of varieties. The differences in values are significant for all factors. Maximum circularity (0.70 cm) was recorded in cotyledon at 50 DAA whereas in seed, its maximum (0.76 cm) values attained at 40 DAA itself and it was more (0.73 cm) in CO 2 than Kerala local (0.68 cm) in both seasons.

#### vi. Elongation of embryo and seed (Table 36 and 37)

Maximum elongation ratio (0.53, 0.70 cm) was achieved at 50 DAA. Thereafter, elongation had a slight decline and attained static till 70 DAA. Maximum (0.54,0.70 cm) elongation ratio of cotyledon and seed was achieved at 50 DAA in rainy season, which was slightly low (0.52, 0.69 cm) in summer season. CO 2 had

more elongation (0.54, 0.70 cm) than Kerala local (0.51, 0.69 cm) with respect to elongation of cotyledon and seed, respectively. During summer, the peak elongation ratio recorded in CO 2 and Kerala local were 0.51 and 0.53 cm for cotyledon, and 0.69 and 0.72 cm for seed. In rainy season, the values were 0.58 and 0.50 for cotyledon and 0.73 and 0.68 for seed in respective varieties.

#### vii. Shape factor in embryo and seed (Table 38 and 39)

Cotyledon and seed of CO 2 and Kerala local had more values during initial stages of growth in both seasons and reduced as growth progressed. The variety CO 2 and Kerala local recorded 0.99 and 1.24 and 1 0.99 and 1.16 during summer and rainy season, respectively for cotyledon. The shape factor values recorded in CO 2 and Kerala local for seeds were 1.01, 1.15 and 1.05 and 1.21 for summer and rainy, respectively.

#### 4.2.29. Gel electrophoresis of total protein

Total extractable protein was examined by SDS-PAGE. From the seed protein banding pattern, it was evident that on set of accumulation of major class of reserve seed protein-globulin 11S was visible from 30 DAA and 40 DAA (lane 3, 4) in both varieties of CO 2 and Kerala local and their concentration was very low. Seed protein bands were distributed into four distinct zones. It was also evident that significant amount of matured moderate molecular weight, as well as low molecular weight proteins, which were synthesized as larger precursors, could be observed from the 40 DAA (lane 4 onwards in both varieties). During the subsequent periods of development, most of the proteins accumulated, showed the pattern of matured (50, 60 and 70 DAA) processed storage proteins (lanes 5, 6 and 7 onwards). There was no major difference of banding concentration during the advance-matured stages after 60 DAA. In matured stages, there was no difference in banding pattern in upper two zones and extra banding observed only in lower two zones in both varieties. High molecular weight proteins were totally absent in both varieties of all

the developmental stages and seasons which was notable finding reported in ash gourd varieties CO 2 and Kerala local.

Identical results were obtained in rainy season in all the developmental stages in both varieties, but they differed in banding and their concentrations during latter stages (60-80 DAA) of seed development. Protein classes having lower molecular weight were predominantly expressed in Kerala local, which was expressed low in CO 2. As far as varietal variation is concerned, Kerala local had extra bands in the lower zones which were expressed predominantly during the later stages of seed development, as this was evident from banding pattern.

# 4.2.3. INFLUENCE OF FRUIT AND SEED POSITION ON SEED QUALITY

## **4.2.3.1.** Seed moisture content (Table 40)

In case of fruit position in the plant, top fruits had lower (30.8 per cent) moisture content; closely followed by bottom (30.9 per cent) and the highest (39.5 per cent) value was recorded in the middle fruits at 70 DAA. Differences were more during the initial period of fruit growth and narrowed down during peak maturity. Among seed cavity segments, stylar segments had lower (33.1 per cent) moisture content and the peduncular had higher (35.6 per cent) moisture, respectively. In all the fruit maturity stages, peduncular segment had highest seed moisture of 40.7, 37.1 and 36.6 per cent at 40, 50 and 60 DAA, respectively.

#### **4.2.3.2. Dry** weight of seed (Table 41)

At 30 DAA, bottom (29.9 g) and middle (24.7 g) fruits had yielded seeds with higher dry weight when compared to top fruits (23.9 g). At final maturity stage i.e., 70 DAA, top fruits attained first position (40.5 g) followed by bottom (37.3 g) and least weight was recorded in middle (37.0 g) fruits. Among fruit segments, stylar had recorded (39.7 g) maximum seed dry weight (39.7 g) and minimum was recorded in peduncular segment (37.0 g).

#### **4.2.3.3. Germination (Table 42)**

Fruits produced at top position had the highest germination of 91.3 per cent, followed by bottom (88.9 per cent) and middle (88.5 per cent) at 70 DAA of fruit maturity. Throughout the fruit maturity stages, seeds from peduncular showed poor performance of 34.4, 84.9 and 87.4 per cent germination when compared to stylar (52.0, 88.8, 89.8 and 90.6 per cent) and intermediate (41.6, 87.7, 89.2 and 89.7 per cent) as per the values recorded at 40, 50, 60 and 70 DAA, respectively.

#### 4.2.3.4. Field emergence (Table 43)

Top fruits had higher (90.1 per cent) seedling emergence when compared to bottom (88.2 per cent) and middle (87.4 per cent) when the fruits harvested at 70 DAA. Seeds extracted from stylar and intermediate segments were found to give better emergence in all the fruit positions which recorded an average per cent emergence of 50.1, 86.9, 88.1 per cent and 89.4 and 39.4, 86.0, 87.1 and 88.9 per cent during 40, 50, 60 and 70 DAA, respectively. Seeds from peduncular segment had lowest emergence of 33.6, 80.0, 83.6 and 87.4 per cent in the respective stages.

#### 4.2.3.5. Dry weight of seedlings (Table 44)

Top fruits had the highest (42 mg) seedling dry weight followed by bottom (39.0) and middle (37.8 mg) at 70 DAA. Among seed segments, stylar segment had maximum value (39.9 mg) followed by intermediate (39.1 mg) and peduncular (37.5 mg). Throughout the developmental stages, seeds extracted from peduncular segment had very low seedling dry weights of 30.8, 34.6 and 37.5 mg at 40, 50 and 60 DAA, respectively.

#### 4.2.4. EFFECT OF FRUIT STORAGE ON SEED QUALITY

## **4.2.4.1. Fruit weight loss (Table 45)**

The fruits subjected to storage, were recorded the initial weight ranged from 4.50 to 5.10 kg and 4.41 to 5.76 kg in CO 2 and Kerala local, respectively. Fruits were lost their weight during the storage from the first month onwards from 3.7 and

2.51 per cent to 52.9 and 49.9 per cent in CO 2 and Kerala local, respectively. The fruit weight per cent loss was slightly more in Kerala local than CO 2.

#### 4.2.4.2. Hundred seed weight (Table 46)

During storage, hundred seed weight had showed slight deviation from that of fruit weight, as both test varieties extended its weight accumulation, which was manifested in hundred seed weight. The hundred seed weight of 5.62 and 9.62 g had initially increased to 6.32 and 9.71 g in CO 2 and Kerala local after fourth and second months of storage, respectively. After twelve months of storage, the hundred seed weight was reduced to 5.00 and 6.15 g, respectively.

#### 4.2.4.3. Seed moisture content (Table 46)

After twelve months of storage, moisture content of the seed gradually reduced from the initial values of 57.9 and 58.4 per cent to 31 and 37.7 per cent in CO 2 and Kerala local, respectively. The reduction was faster in CO 2 than Kerala local. Maximum reduction in moisture content was observed at fifth (41.5 to 38.8 per cent) and eighth months (50 to 47.4 per cent) after storage in respective varieties. Thereafter, both varieties had faster reduction in moisture content till the end of twelve months of storage.

#### 4.2.4.4. Fresh seed weight (Table 47)

At the time of storage CO 2 recorded 81.3 g and Kerala local recorded 160.3 g of fresh weight during storage upto third and second (84.3 and 183.3 g) months. The reduction started at fourth month and seventh month in CO 2 and Kerala local, respectively (80.1 and 180.3 g). At 12<sup>th</sup> month, the decrease in weight of seed was 62.3 and 162.3 g fruit<sup>-1</sup>, respectively.

## **4.2.4.5. Dry** seed weight (Table 47)

As the storage period increased, the dry weight initially increased from 125 to 126.2 and 50.6 to 56.9 g in second and fourth months of storage in CO 2 and Kerala

local, respectively. The final weight recorded in CO 2 and Kerala local were 50.5 g and 111.4 g in respective varieties.

#### 4.2.4.6. Speed of germination (Table 48)

Very low speed during initial, first and second months of storage in CO 2 (10.2, 10.9 and 12.1) and Kerala local (3.2, 4.6 and 5.7) was observed. The maximum speed of germination was recorded in eighth month (14.5) in CO 2 and ninth month (13.2) in Kerala local. Afterwards, the values showed a slight reduction over a period of storage.

#### **4.2.4.7. Germination (Table 48)**

At the time of storage, the germination was 36.5 per cent in CO 2 and 37.8 per cent in Kerala local. Over a period of storage, the germination increased to 94.5 and 91.5 per cent eighth months of storage. Afterwards, a decrease in germination was noticed till the end of storage.

#### **4.2.4.8.** Shoot length (Table 49)

The increasing trend over a period of storage was also noticed for shoot length recording 18.3 cm at 7<sup>th</sup> month in CO 2 and 21.6 cm in Kerala local at 7<sup>th</sup> month of storage. Thereafter, the shoot length had decreased values in both the varieties.

#### **4.2.4.9.** Root length (Table 49)

Root length increased from 10.8 cm (0 month) to 15.3 cm (6<sup>th</sup> month) in CO 2 and from 12.0 cm (0 month) to 19.6 cm (8<sup>th</sup> month) in Kerala local. Decline was maximum in CO 2 than Kerala Local during the remaining period of storage.

## 4.2.4.10. Dry weight of seedling (Table 50)

As the fruit storage period increased, there was also an increase in seedling weight. The maximum seedling weight was recorded at fifth months after storage in CO 2 (38.5 mg) and in Kerala local (42.9 mg) at 6<sup>th</sup> month after storage. After

reaching the peak seedling weight, a declining trend was noticed till the end of storage.

#### **4.2.4.11. Vigour index (Table 50)**

The initial vigour index of 807 increased to 2957 at sixth months of storage in CO 2 and from 873 to 3733 in Kerala local at seventh months of storage. The final vigour index recorded at 12<sup>th</sup> month of storage was 1779 in CO 2 and 2823 in Kerala local.

# 4.2.5. STANDARDISATION OF A SUITABLE METHOD OF SEED EXTRACTION, SEPARATION WASHING AND DRYING

#### 4.2.5.1. Speed of germination (Table 51)

Regarding the method of seed extraction, closed fermentation was the best compared to non fermentation and open fermentation, which recorded a higher speed of germination of 12.4, followed by open fermentation (11.0) and non fermentation (7.5) at first month of storage. Similar trend was maintained throughout the storage period except fifth month of storage. At fifth month of storage, both closed fermentation (14.3) and open fermentation (14.6) had equal performance, which was superior to non-fermentation (10.7). During the remaining period of storage, closed fermentation outperformed other methods of fermentation. Among different combination of drying and washing methods, combined process of washing 15 minutes, drying at room temperature for 25°C followed by sun drying as well as washing + sun drying recorded 10.4 and 10.5 speed of germination, which were superior than values recorded in sun drying (9.2) and drying under room temperature + sun drying (9.7).

#### **4.2.5.2. Germination (Table 52)**

Closed fermentation had higher germination (87.5 per cent) when compared to open (77.3 per cent) and no fermentation (60.9 per cent) at first month of storage. Similar trend was observed in all the storage period. At fifth month of storage, closed

fermentation had as high as 93.1per cent germination, which was slightly lower than open fermentation (94.5 per cent) and superior to no fermentation (86 per cent). Combined process of washing, drying at room temperature + sun drying had higher germination of (87.2 and 92.9 per cent) in first and fifth months of storage respectively followed by washing + sun drying (82.4 and 90.7 per cent) and very lower germination was reported in sun drying (72.8 and 88.1 per cent) during first and final storage periods.

#### **4.2.5.3.** Shoot length (Table 53)

During first month after storage not much variation was observed among different extraction methods. As the storage period increased closed, fermentation outperformed open and non-fermentation for shoot length of seedlings. At fifth month of storage, closed fermentation outperformed others by recording 20.4 cm of shoot growth against 18.3 cm (open fermentation) and 17.2 cm (non-fermentation).

Equal performance of shoot length was observed in the seeds received the treatment of washing + sun drying at room temperature and washing + drying at room temperature and sun drying at first month of storage (12.5 cm). Similar trend was maintained in all the storage periods. Sun drying and drying at room temperature + sun drying had poor performance by recording 11.2 and 11.7 cm shoot length during the first month and 18.4, 18.1 cm at fifth month of storage when compared to other treatments.

#### **4.2.5.4. Root length (Table 54)**

Closed fermentation recorded a clear lead of 10.9 cm of root growth followed by open fermentation (10.5 cm) and non-fermentation (10.0 cm). Similar result was maintained throughout the storage period. Regarding drying and washing methods, except combined treatment of washing + drying at room temperature + sun drying (11.2 cm), all other treatments had poor performance by recording low root length in all the storage period. However, at final month of storage, the differences among

treatments were reduced and they recorded 12.4, 12, 12.5 and 12.5 cm in the sun drying, drying at room temperature + sun drying, washing + sun drying and washing + drying at temperature + sun drying methods, respectively.

# 4.2.5.5. Dry weight of seedling (Table 55)

At first month of storage, open fermentation (38.4 mg) and closed fermentation (37.1 mg) were superior against non-fermentation (28.9 mg). As the storage period increased, closed fermentation had a clear lead against other methods and at fifth month of storage, closed fermentation recorded as high as 42.8 mg closely followed by open fermentation (42.4 mg) and non fermentation (34.7 mg).

At initial storage periods, except sun drying (32.8 mg) all other treatments had a more or less equal performance by recording 35.8, 35.5 and 35.2 mg of seedling dry weight due to washing + drying at room temperature + sun drying, washing + sun drying, and drying at room temperature + sun drying and sun drying respectively. However in all the storage period, washing + drying at room temperature + sun drying showed a significant difference over other treatments.

#### **4.2.5.6.** Vigour index (Table 56)

Closed fermentation was the best method (2124) compared to open fermentation (1917) and non-fermentation (1675) at initial storage itself. Similar result was maintained in all the storage period. At fifth month of storage also, closed fermentation outperformed others by recording 2781 against 2650 (open fermentation) and 2183 (non fermentation control).

In all the storage period, combined washing + drying at room temperature + sun drying treatments outperformed other treatments by recording 2201 and 2668 of vigour index value respectively during first and final (5 months) months of storage. Whereas control had a vigour index value of 1646 and 2313 during the respective period of storage.

# 4.2.6. ON FARM TRIAL TO TEST VERIFY THE BEST METHOD OF SEED EXTRACTION, SEPARATION, WASHING AND DRYING

From the previous study, it is inferred that closed fermentation for 72 h and washing for 15 min. drying under room temperature (25°C) for 72 h and sun drying for 12 h was the best by recording higher germination (95.5 per cent) and vigour (2668). Hence, this method of seed extraction was test verified in the ash gourd seed producer's unit at M/S K.R.Rangaraj, Kondamuthunur, Sathyamangalam (Taluk), Aryappanpalayam-638452, Erode (District), Tamil Nadu. The two acre ash gourd seed crop yielded 60,100 kg of fruits after harvest. Out of 60,100 tonnes, after sorting (15,100 kg) malformed, under sized and bruised fruits, 45,000 kilogram of fruits was subjected to this method of seed extraction. The total cost of cultivation was worked out to Rs. 20,000/-. Seed extraction alone had incurred an expenditure of Rs. 17,275/-for 45,000 kg of fruits with 0.68 per cent good seed recovery.

#### **CHAPTER V**

## DISCUSSION

"Seed is the quintessential symbol of our self-reliance and the legacy of our agricultural heritage. The changes in weather condition have adverse effect on seed quality and thus, have important consequences for the role of plants in bio-regenerative life support systems, as well as implications for dynamics within the microenvironment of the developing seed" (Musgrave, 2002).

In our condition, manipulation in weather condition is difficult instead, we are contemplating on factors like time of sowing, stage of harvest and post harvest handling to achieve production of quality seeds. In this direction, experiments were conducted in ash gourd varieties, CO 2 and Kerala local to standardize some of the practical seed production aspects related to flowering behaviour as influenced by varieties and seasons, changes during fruit and seed development and maturation, and position of fruit and seed, fruit storage and seed extraction methods on seed quality, at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. On Farm Trial for test verification of the best method of seed extraction, separation, washing and drying was conducted at seed producers unit, Kondamuthunur, Sathyamangalam taluk of Erode district. The results obtained from the experiments are discussed in this chapter.

# 5.1. FLOWERING BEHAVIOUR AS INFLUENCED BY VARIETIES AND SEASON

Significant influence of season on flowering behaviour was observed in ash gourd and it was also affected by variety. The delay in flower initiation, anthesis, wider sex ratio were the disadvantageous aspects faced by crop raised in summer. In contrast, rainy crop had all the advantages, because of prevailing suitable weather conditions especially adequate rainfall (471 mm), rainy days (26), average temperature (23.8 – 27.5°C), RH (84 to 92%) and solar radiation (350 to 424 ca cm²/d), which culminated in the production of more number of fruits per vine. Weather data recorded during the study period are presented in the Figure 3a, 3b, 3c and 3d.

In both the seasons, the superiority of variety CO 2 over Kerala local was clearly evidenced in the flowering behaviour, except number of fruits per vine. Kamalanathan *et al.* (1972) reported that short day condition with 29.4-29.9°C favours femaleness in ash gourd under Coimbatore condition. More and Munger (1987) observed narrower and wider sex ratio in the rainy and summer crops of muskmelon, respectively. Likwise, Desai and Patil (1985) obtained lower male to female ratio in watermelon variety, Sugar Baby raised in rainy crop (July) than a crop sown on January month.

The present experimental results indicated that inspite of adverse weather condition of high temperature (24.7 to 30.2°C), and inadequate rainfall (108 mm), ash gourd could be established successfully and produced satisfactory number of fruits during summer season. This has proved the characteristics adaptability of ash gourd. But their growth rates are significantly altered due to the season (Nath and Vashistha, 1970). According to Kamalanathan and Thamburaj (1970), pre-flowering and flowering phases in *Cucurbita moschata* were governed mainly by photoperiod and temperature and not affected by humidity and rainfall. But in the present experimental field, rainfall during March, 2002 (69.2 mm) actually boosted the growth of the plants during summer season and that has helped for successful crop.

The present investigation clearly indicated that sowing during the month of July (rainy season) were the best by recording low sex ratio of male to female flowers with maximum numbers of fruits per vine in ash gourd.

# 5.2. FRUIT AND SEED DEVELOPMENT AND MATURATION AS INFLUENCED BY VARIETIES AND SEASONS

In fleshy fruited crops, since seeds are developing inside the fruit, maturity status of both fruits and seeds must be taken into account to obtain maximum quality of seed with full mass maturity, indicated by maximum dry weight, germination and vigour.

To ascertain the correct stages of fruit maturity for maximizing the yield of high quality seed, development and maturation of fruits and seeds were studied in ash gourd varieties CO 2 and Kerala local by growing them in rainy and summer seasons.

Fruit weight, circumference, length, diameter and pulp thickness of ash gourd were found to increase from anthesis to maturity. Regarding fruit weight, it was maximum at 60 DAA in summer season and 70 DAA in rainy season.

During initial growth stages, differences among varieties and influence of season were quite low for the fruit characteristics. The summer crop of both CO 2 and Kerala local produced fruits with smaller diameter when compared to rainy season. The differences in fruit diameter observed between summer and rainy season was 3.5 cm in CO 2 and 1.7 cm in Kerala local at 60 DAA.

Most striking varietal difference observed between CO 2 and Kerala local was the seed cavity. The former had lower diameter with a range from 8.5. to 8.6 cm with large pulp thickness (15.7 to 16.5 cm) which the later possessed higher diameter of seed cavity (11.7 to 11.9 cm) and lower pulp thickness (7.6 - 7.7 cm) during the mature stages of 60-70 DAA.

Good seed recovery was obtained during the later stages of seed development in both seasons and varieties after mass maturity of 55 DAA onwards. Rainy season had 1.18 per cent and summer season recorded more or less equal value of 0.97 per cent as number of filled good seed fraction was high during these stages. Not much variation was observed for fruit characteristics *viz.*, seed cavity and pulp diameter between the seasons.

The variety CO 2 was characterized by producing as high as 9.0 kg of fruit weight and Kerala local found to produce medium size fruits (5.0 kg) with proportionate in the values of other parameters. In rainy season, fruit continues to grow actively as long as it is attached with vine (80 DAA). In summer, the fruit size and weight was culminated much early in 60 DAA and then declined slightly. Balakrishnan *et al.* (1986) obtained increased growth in fruit characteristics as the age of fruits advanced in fleshy fruited papaya. Maximum fruit weight, length and number of seeds per fruit were obtained at 18 and 24 DAA in okra by Somnotra *et al.* (2002) in spring-summer and rainy season, respectively.

With respect to seed, the maximum dry mass accumulation occurred much earlier than fruit (55 DAA) in both seasons. This is in agreement with the findings of Berry and Bewley (1991) in tomato and Jing *et al.* (2000) in and cucumber seeds who reported a poor relationship between fruit and seed development. The reason attributed for continuous weight increase in fruit was moisture accumulation in the fruit pulp, which was restricted in seed beyond its maximum accumulation of dry weight. The amount of water taken by the seed is also a good indicator of seed maturation and germinability and immature seeds take considerably more water than mature seeds (Nerson and Paris, 1988).

Except seed moisture content, all the seed parameters had an increasing trend as age of the ash gourd fruit increased. The maximum fresh and dry weight (mass filling stage) occurred at 55 DAA in summer and it was extended upto 60 DAA in rainy season. In subsequent stages of development and maturation, there was no much change occurred in rainy season for weight of both fresh and dry seed, whereas in summer season drastic reduction of fresh weight was observed (Figure 4a and 4b).

Accumulation of fresh and dry weight of seed was more in Kerala local than CO 2 in all the stages. The varieties CO 2 and Kerala local had accumulated 60 and 72 per cent of drymatter from 20 DAA to mass filling stage in summer season. Whereas, in rainy season both varieties had the highest values of 32 and 60 per cent respectively. These results in ash gourd are tallying with the suggestion of Ellis and Pieta Filho (1992), who described the turning point of maximum dry weight as mass maturity, which was called previously as physiological maturity. Because seed at this point though accumulated maximum dry weight, they are not mature enough to give potential longevity, germination and germination rate. Dry mass accumulation has substantially occurred in developing cotyledon, which is reflected of the measured increase in the cotyledon weight and thickness as reported by Jacks (1990) in cucurbit seeds. Beyond mass maturity, cotyledon had slight increase in accumulation which is negligible when compared to initial rate of thickness and weight increase during mass filling.

The loss of moisture content of seed with time was linear over the range from 59.0 to 43.0 per cent in summer season and 70 to 49 per cent in rainy season during

initial, 20 DAA to mass filling stage, respectively (Figure 4a and 4b). When compared to other cereal crops, reduction in moisture content was not substantial over the period of time as seeds mature and remained within a relatively moist environment in fleshy fruit, where desiccation is prevented or delayed (Welbaum, 1999). In tomato and pepper, the moisture content of mature seeds is maintained at a comparatively high value within the moist fruits. This is more or less similar to all fleshy-fruited crops (Demir and Ellis, 1992a and b). Seed moisture content at maturity was 46 per cent in marrow and it was stabilized to 40 per cent from about 65 DAA onwards (Demir and Ellis, 1993). In this experiment, summer and rainy season fruits had 43-44 per cent and 49-50 per cent of moisture content during the mature stages, respectively.

Germination was initiated in ash gourd at 30 DAA when it was raised in summer, which was approximately 30 days before mass maturity. Semi permeable characteristics of 'cellose', a single layered suberised cover deposited on the outer surface of the endosperm exert resistance to on set of germination during the earlier phase of seed development, which was reported by Yim and Bradford (1998) in muskmelon which is present in ash gourd seed also. Proportion of seeds showing a positive staining in tetrazolium viability test was zero at 30 DAA and increased rapidly to 97.3 per cent in rainy season at 80 DAA and 88.5 per cent in summer season at 65 DAA. This is exactly in agreement with the results obtained in marrow during seed development and maturation by Demir and Ellis (1993). In rainy season, seeds from stage 30 DAA has failed to initiate germination. Muskmelon embryo develop the ability to germinate sequentially well before the accumulation of maximum dry weight, which occurs roughly 35 DAA (Welbaum, 1999).

At 35-55 DAA, moderately mature fruits of ash gourd may disguise the harvester to make mistake for mature one, since entire fruit rind at this stage was covered with ash coat. Hence, once over harvest consisting of mature and immature fruit results in fall of total germination. Seed lots of this kind may not pass minimum seed certification standard (MSCS) of 60 per cent germination in ash gourd. This is the exact reason why there are frequent complaints from seed certification and seed testing authories that seed samples of most of gourds are often failed to pass the minimum seed certification standard for germination.

In summer season, germination initiated from 40 DAA onwards and reached the highest, after mass filling stage and continued to increase till 65 DAA. Rainy season crop maintained the high level of germination upto 80 DAA, whereas in summer it was maintained upto 70 DAA only. At 60 DAA, both CO 2 and Kerala local surpassed the maximum certification standard of 60 per cent germination, irrespective of growing season. During this stage pedicel, of the fruit became dark green, angular, ash coat became powdery and peduncular shoulder depressed to the maximum. The higher germination percentage, vigour index and optimum seed moisture were observed at 27 and 36 days after anthesis in spring summer and rainy season crops, respectively (Saminotra *et al.*, 2002)

There was a little variation occurred among two varieties at this stage (60 DAA) and differences were nullified in subsequent stages for germination. So, harvest may be postponed until last maturity beyond 80 DAA in rainy season and 70 DAA in summerseason. Delay in seed harvesting did not result in substantial decline in potential longevity (Demir and Ellis, 1992) in tomato Demir and Ellis (1993) also observed increased potential longevity, germination and germination rate beyond maximum dry weight accumulation in vegetable marrow.

Ash gourd seeds maintained a near maximum or normal germination for 20 days in summer and 30 days in rainy season from 50 DAA to 70 DAA and 80 DAA respectively. During this period, seed moisture content within the fruit was maintained at values close to 50 per cent which was much higher than the values recorded in mature cereal seeds such as barley (Ellis and Pieta Filho, 1992) and pearlmillet (Kameswara Rao *et al.*, 1991). In fleshy fruited crops, maturation occurs normally after the end of natural seed filling phase and during the pre-harvest maturity period seed deterioration is prevented within the fleshy fruits due to cellular repair and turn-over process. This was reported in tomato by Demir and Ellis (1992) and Kwon and Bradford (1987).

Villiers and Edgecumbe (1975) had proposed the hypothesis that seeds held at moisture content close to full imbibition were provided with free oxygen, but germination was prevented and seeds able to maintain their viability for considerable periods. They postulated that this was because of cellular repair and turn over

activities occur within the seeds, under such conditions, such that any damage that might otherwise accumulate during storage is rapidly repaired. Members of cucurbit crop avoid germination inside the fruit for long time due to the accumulation of osmotic solutes, which creates low water potential inside the developing fruit that maintains seed water potential below the threshold required for germination (Welbaum *et al.*, 1990)

Thus, Villiers hypothesis can also be applied to pre-harvest storage of ash gourd in order to explain the maintenance in maternal environment for 20 to 30 days to get high quality seeds even after achieving mass maturity at 55-60 DAA.

Seed moisture content in the developing and mature fruits of ash gourd remains high throughout the development and maturation. So in fleshy fruited crops, maximum germination was achieved several days after mass maturity as reported in many crops. In contrary, Doijode (1983) and Doijode (1988) obtained maximum germination some days before mass maturity or just after mass maturity in tomato and chilli.

With respect to laboratory germination test, maximum shoot length occurred at 5 and 15 days after mass filling stage in summer and rainy seasons, respectively which coincide with 60 DAA in summer and 70 DAA in rainy season. Similar results were also obtained in case of seed growth and seedling dry weight in tomato by Argerich and Bradford (1989) and Ellis (1989) in onion, respectively.

In the present investigation, a positive correlation was obtained between seed maturity, and germination, shoot and root length, seedling dry weight and vigour index. Fruits harvested beyond 60 and 70 DAA in summer and rainy season respectively yielded quality seeds that showed good strength of physiological parameters *viz.*, germination, shoot and root length, seedling dry weight and vigour index (Plate 9a and 9b). Similar pattern of seed development and maturation was also obtained by Krishna Prasad (1980) in ash gourd cv. CO 1, Bejoy Kennath and Devdass (1996) in BH 21 ash gourd, Hedayat (1987) in watermelon and Adirai (1999) in pumkin.

To conclude this study, seed quality in ash gourd remained high after mass maturity (55-60 DAA) and attained maximum quality which continued upto 70 DAA in summer and 80 DAA in rainy season. In practice, seed producers do not leave fruits on the plant for an expanded period (70-80 DAA) because of the likelihood of viviparous germination and disease or damage by pest. From the results of physiological evaluation, it would appear sensible to suggest that fruits can be harvested 60 DAA in summer season, when pedicle become dried or angular, and 70 DAA in rainy season, when fruits become thick with powdery ash and peduncular end of the fruit shoulder depressed fully. Leaving fruits beyond 80 DAA in rainy season invites fungal growth. During the final maturity stages, seed coat became dark brown detached from the placenta, easily comes out from the pulp when cut open and scooped (Figure 6 and 7). At 70 DAA, almost all the fruit pedicel portion of summer crop became dried (Plate 10a and 10b). Complete crop senescence occurred 170 days after sowing in summer, whereas in rainy season vine and fruit attachment with plants existed beyond 180 days.

Important biochemical changes during seed development was estimated for different stages of seeds from 30 DAA to penultmate maturity. Overall results obtained proved that seed maturity starts from 60 DAA in summer and 70 DAA in rainy season. The measured increase in the thickness of the embryo reflects an increase in the storage reserves especially protein and lipid as reported by Jacks (1990) in cucurbits. Significant difference was not obtained for dehydrogenase enzyme activity and oil content of the seed in the present study. However, numerical increase in values was obtained in the mature stages over immature seeds. The accumulation of protein followed more or less sigmoidal pattern in ash gourd seed, which started from 30 DAA and continued upto 70 DAA in summer season while rainy crop had protein accumulation beyond 70 DAA and continued upto 80 DAA. However, after 50 DAA, accumulation was stablised. Similar pattern of protein and oil accumulation was recorded in developing seeds of brassica by Dasgupta and Mandal (1993).

#### **Histological studies**

#### **Seed coat development**

Structural changes in seed coat during seed development and maturation examined using microtome sectioning are presented in Plates 11a to 11c.

According to Dathan and Singh (1990) seed coat development in cucurbit is peculiar during development and maturation of seed. It was evident from the microtome sections that structural changes in seed coat of mature seeds were entirely different from the seed coat of immature seeds. The seed coat of over mature seed was visible as non-structured cell walls and at the peak maturity stage, multiple layers of cell wall was visible with maximum expansion. Elongated tubular or rod shaped thickening in epidermal cell running in the radial direction from the inside to outside, was visible in the seed coat of mature seeds of ash gourd, which was absent in the seed coat of immature seeds. The same structure with different shape was observed in Cucurbita pepo by Singh (1953). In immature seed, seed coat layers were so compact that it was difficult to split by growing embryo. Similar finding was expressed by Edelstein et al. (1995) and Bewley and Black (1982) in muskmelon, and they opined that seed coat and other surrounding structures may influence germination by exerting mechanical restriction to embryo growth. In seeds of melon, the embryo was enclosed by a testa and a thin perisperm, that were permeable to water (Welbaum and Braford, 1990). In the present study, the testa got thickened as age of seed increased and it was stopped ahead of cotyledon expansion.

#### Embryo development

The structural changes in embryo during the seed development and maturation through microtome examination are depicted in the Plates 12a (i to vii).

Like most dicotyledonous species (Raven *et al.*, 1992), embryo morphology in ash gourd seed during embryogenesis proceed through pre-embryo and globular at early cotyledon stages and enlargement in the middle stages (30-50 DAA) and filling and maturation in the late stages (60-80 DAA). Ma and Zheng (1992) have classified into stages of the embryo development in Chinese cabbage seeds according to its

morphology and they recorded embryo development from 5 to 10 DAA as a single stage. Kuang *et al.* (1996) divided *Arabidopsis thaliana* embryo development into six stages as late globular, cotyledon initiation, early linear cotyledon stage, late linear cotyledon stage, cotyledon stage and mature embryo.

In general, seed development was divided into embryo cell division and differentiation, rapid accumulation of storage materials and dehydration as per the findings of Dasgupta and Mandal (1990) observed in *Brassica* seed. Accordingly, in ash gourd, maximum expansion of cells was observed in last maturation stage (60 DAA) which came under second phase as per dicotyledenary seed development. In ash gourd, this second phase was either delayed or extended even after attaining full maturity. During the intermediate development stages (30-50 DAA), rapid accumulation of storage materials was observed. During the initial development of cotyledon (20-25 DAA), cell division was completed which was evident from the sections taken from the developing, immature and young seeds (20-30 DAA). Aaccording to Yim and Bradford (1998), muskmelon seed also stopped their cell division during 30-35 DAA.

In the present study, the cotyledons developed the ability to expand first (20 DAA), followed by embryonic axis development (30-60 DAA). During embryonic axis development, hypocotyls expanded first, followed by root and shoot meristems. This is similar to the results observed in muskmelon seeds by Welbaum and Bradford (1989).

In this present study, late globular stage occurred approximately at 5 DAA. Early stage of cotyledenary development was observed at 15 DAA. At 20 DAA, complete cotyledenary structure had developed. During the subsequent stages (upto 50 DAA), cotyledon thickening and embryo differentiation took place. Mature embryo could be observed beyond 50-60 DAA. From over mature seed stage (80 DAA), radicle cells (Plate 12b (i to vii) were highly enlarged and multiple growth

was observed in hypocotyl axis. Cell enlargement in all other stages was proportionate to their increased growth stages.

#### Measurement of seed and embryo descriptors using image analysis system

The developing and maturing seeds and embryo were subjected to image analysis systems for observing the variation in the physical characteristics.

This method had broad potential usefulness as a rapid indicator of seed lot quality. An appropriate integration between automated machine vision techniques and standard seed evaluation tests could provide more detailed information on the status of seed lot (Heijden *et al.*, 1999). The image analysis method was used to measure ash gourd seed size and shape based on perimeter, area, elongation, circularity, length, width and shape factor. All the parameters were found useful in fixing the pattern of the maturity status of seed. The data obtained were also used to findout the difference in the distinguishable seed morphological characteristics of CO 2 from Kerala local (Figure 8a and 8b). According to Vooren *et al.* (1991), crop varieties can be identified based on shape descriptors like area, perimeter and shape factor. They have identified the parameters showing significant differences. According to Drzewiecki *et al.* (2000), size and shape of the seeds are typical of a species and variety.

In rainy season crop the study of seed characteristics of both the varieties using image analysis system showed that area, perimeter, length and width of the cotyledon as well as continuity of seed growth reached the highest values at 70-80 DAA, which was coincided with the stages after mass filling maturity stage. Most significant point observed here was that there existed of a varietal variation, between the two varieties for all the stages studied starting from 20 DAA to 80 DAA.

The variety Kerala local had registered higher geometric values listed above, indicating bigger size seeds, when compared to CO 2. Except numerical difference,

cotyledon and embryo showed the similar trend with respect to growth and development. Very high variation was observed with regard to width of seed and cotyledon. Correct and accurate varietal identification was possible based on width or shape factor as suggested by Drzewiecki *et al.* (2000). Many seeds are similar in shape, but differ widely in size. Size is often used in description of plant seeds in order to assess identification (Shieh and McDonald, 1982). Significant finding observed here were the elongation and circularity values of cotyledon and seeds, which were more in the initial growth stages (30 DAA) and as the growth increased, the valves were also stabilised. All the measured valves of seed shape factor exceeded 1.0, which is the maximum theoretical value expected. It is a feature of the picture element and does not affect the utility of data. At initial stages (30-40 DAA) of seed development and maturation, shape factor value was higher than mature (50-70 DAA) stages.

The conclusion from this study is that by using machine system a good varietal discrimination could be established and growth of objects could be monitored for maturity status of the seed during development and maturation. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local confirmed their maturity stages, as both cotyledon and seeds of mature stages observed to have more values of seed descriptors. According to Edwards and Lowe (1981), image analysis method can be used apart from capturing binary images and subsequent measurements of size and shape in relation to germination and growth performance in laboratory and field.

Positive relationship was observed for growth stages with seed descriptor values and seed performance in terms of germination. Seed descriptors using image analysis system could be done very quickly and large volume of samples could be subjected to observation within a shorter period of time.

#### Gel electrophoresis of total protein

Gel electrophoresis of protein at different stages of ash gourd seed development revealed that occurrence of mature and processed storage proteins in the bands representing seed stages of 40 to 70 and 80 DAA in summer and rainy season crops, respectively. In the initial stages (20-30 DAA), very less accumulation with lower contractions was noticed. High molecular weight proteins were totally absent in both varieties of ash gourd, which could be observed in all the lanes of both seasons (Plate 13). Pasha (1986) and Pasha and Sen (1991) have examined the electrophoretic patterns of seed protein in mature seeds of cucurbits and detected 3-5 bands having moderate molecular weights, which contributed major fractions.

In the present study, identical banding pattern was reflected in both summer and rainy seasons, but varietal variation observed as Kerala local showed extra banding especially in the less molecular protein area (lower zones among four zones) in 20 and 14.3 Kda. Expression was high at 20 Kda than 14.3 kda. Pasha and Sen (1989) reported difference of protein band having lower molecular weight in *Cucumis melo* var. *melo* and var. *agrestis*. The major reserve seed proteins reported in cucurbits seeds was 11S globulin (Guha and Sen, 1998) which was expressed in this study at 20 kda and adjoining flow area 2S globulin was expressed in 14.3 kda.

Crouch and Sussex (1981) found that onset of storage protein accumulation in *Brassica* sp. coincided with the beginning of phase two of embryo growth which was roughly 30 DAA in ash gourd as defined in histological studies. In contrary, Norton and Harris (1975) showed that major proteins found in the mature seeds of *Brassica* do not appear in the initial stages of seed development. This is in agreement with the results obtained in this study, as initial stages of ash gourd showed very less protein concentrations in the form of bands. During the first phase of cotyledon development, cotyledon was small part of the seed and its proteins would thus be diluted (Norton *et al.*, 1976). Since, the whole seeds were used for gel electrophoresis, very light concentrations of bands were obtained in this study. Norton *et al.* (1976) also expressed similar view.

From the electrophoresis study, it is concluded that precise maturity detection is not possible as mature processed proteins exhibit same bands all along starting from 40 to 80 DAA in rainy season, and 4 to 70 DAA in summer season. As per the physio-biochemical studies, stages ranged between 40-50 DAA were moderately mature and putforth very less germination. There was no striking difference with respect to banding pattern between fully mature seeds (60-80 DAA) and moderately mature seeds (40-50 DAA). However, by and large immature stages (20-30 DAA) could be very well differentiated from that of moderately mature to mature stages. This study is very much useful for varietal identification and helpful as basic information for molecular level investigations in ash gourd in future

#### 5.3. INFLUENCE OF POSITION OF FRUIT AND SEED ON SEED QUALITY

There was a significant variation for seed moisture content, seed dry weight, germination, field emergence and seedling dry weight due to position of fruits and seed. At peak maturity of 70 DAA, bottom and top fruits had more or less equal seed moisture content (30.9 and 30.8 per cent), whereas middle fruit had much higher (39.5 per cent) moisture content. Peduncular segment in all the fruit position showed higher moisture content in all the stages (Figure 9a). Such difference in moisture content was also observed in marrow, which occurred 65 DAA, as per the report by Demir and Ellis (1993).

In ash gourd, dry weight of seed increased steadily and reached a maximum at 70 DAA except for peduncular segments, in which the increase in seed dry weight was not encouraging which recorded low dry weight in all the stages. Same differences continued upto 70 DAA with little variation than earlier stages. During the initial stages (30 DAA), basal fruit was the best, recording higher seed dry weight compared to middle and top fruits. But as the maturity stages increased, top fruits had more seed dry weight than remaining position.

Khan and Passom (1992) obtained higher fruit weight and lower seed content in bottom fruit of sweet pepper. Similar increase in seed weight of top fruits was obtained by Jambhale *et al.* (1988). Thus, it may be worthwhile to harvest fruits from top of the vine for obtaining higher seed recovery. Among different segments,

peduncular had very low seed weight. Severe competition occurs among reproductive sinks, maternal resources whose relative developmental stages and position on mother plant influence seed performance (Watson and Casper, 1984; Rocha and Stephens, 1991). The results confirmed the variation in seed dry weight, germination and emergence as observed in the present study. Ash gourd seeds like other cucurbits, acquired germinability prior to the attainment of mass maturity and initiation of seed germination started at 30 DAA from top positioned fruits. This has confirmed the previous findings (Nerson and Paris, 1988) that the process of germination does not require completion of seed development. After 40 DAA, seeds from three positions showed differences in the accumulation of dry weight and germination percentage. Hence, there was a difference in seed quality in the early maturity stage prior to mass maturity, which was revealed by germination and field emergence Nerson and Paris (1988) had a opinion that for initiation of germination, cucurbit seed does not need full seed development. Seed quality variation due to position is in agreement with the performance of tomato and muskmelon seeds observed by Welbaum and Bradford (1989), Berry and Bewley (1991) and Demir and Ellis (1992).

In cucumber also, similar results were reported by Nerson (1991). Seed from various position achieved maximum germination and produced normal seedlings, but showed different values. Seeds from peduncular segments of bottom, middle and top produced low germination. In ash gourd, seeds closer to the stem exhibited lower performance. Seeds from top fruits outperformed middle and bottom fruits (Figure 9b). The mature seeds from top fruits was heavier than those from middle and bottom. This is in conformity with the result of tomato reported by Demir and Ellis (1992), who stated that seeds from the upper truss exhibited good performance than seeds from the lower truss.

The observed lower seed quality within the position might be due to dominance effects of top fruits over the others (Marcelis, 1993). The poor performance from the peduncular segment of a fruit might be a result of fertilization with slow germinating pollen, which might in turn show low germination and vigour than that of fertilizing ovules at the stylar end of the overy (Lau and Stephenson, 1993). In contrary, Doijode (1990) and Doijode (2000) obtained best quality seeds from peduncular segment of the chilli and brinjal fruits. This variation might be due to

the pollination behaviour as these crops are self pollinated and fertilization effect might not influence seed performance like in ash gourd, which is a highly cross pollinated crop.

In ash gourd, variation due to position of fruits and segments exhibited more during the early stages, it prolonged upto 60 DAA. After attaining 70 DAA, variation in seed quality was less. Overall results revealed that performance of seeds from 70 DAA was excellent when compared to other stages. Hence, prolonged maturation of seeds inside the fruit is beneficial for seed quality point of view. This study also confirmed that harvesting of fruits at 70 DAA is a correct and suitable stage for best seed quality in rainy season crop.

From this study it is very clear that in species with linear arrangement of seeds within the fruits, seed position may influence seed quality, which is affected by gametophytic selection. Ovules present in the peduncular segment had lesser opportunity for fertilization than stylar segment, that is why the performance of seeds from peduncular portion is less. In predominantly cross pollinated crop having linear seed arrangement, the influence gets multiplied.

Fruits borne in the early nodes may have advantage of better fruit growth and seed weight increase in the initial stages, as growth of plants progress, the source sink resource will be diverted to seed and in many of the large fruited crops low seed weight was reported. In the apical portion of the plants, fruit size may increase or decrease, but seed content will be better, consequently more seed recovery also. From this study, it is concluded that the ash gourd fruits from the top nodes (beyond 47<sup>th</sup> node) could be utilised for seed purpose and from the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised for vegetable purpose.

#### 5.4. EFFECT OF FRUIT STORAGE ON SEED QUALITY

It is a general practice that gourd fruits are harvested at vegetable maturity and stored for seed extraction in the farmers holding. But leaving the fruits in the vine for full maturity is more effective according to Nerson (1991). Besides the initial germination, longevity of such seeds harvested at the time of vegetable maturity is also poorer than those mature on the mother plant itself. In practice, seed growers may

not take risk in retaining fruits in the field. In this context, storage of fruits is highly essential for quality seed production point of view.

Pre-extraction storage of harvested fruits improves germinability in many cucurbits (Wallerstein *et al.*, 1981; Alvernga *et al.*, 1984). Hence, influence of extended storage of mature (70 DAA) ash gourd fruits under room storage condition was studied using CO 2 and Kerala local varieties. During the course of storage, there was continuous weight loss in both the varieties upto 12 months period due to water evaporation, which resulted in dehydration of fruits leaving pithy pulp and seed mass intact. The extend of weight loss was 53 and 49 per cent recorded in CO 2 and Kerala local, respectively (Figure 10a). The moisture content of the seed decreased from 57.9 and 54.4 to 31.0 and 37.0 per cent, respectively. The loss of fresh and dry weight of seed was reported to be 23.4 and 10.2 per cent and 5.3 and 10.8 per cent in CO 2 and Kerala local, respectively. The weight loss of 4 per cent after three weeks of storage was recorded in muskmelon under room condition at 15°C (Miccolis and Saltveit, 1995). They also reported varietal variation in per cent weight loss.

Germination of seeds significantly increased from 36.5 and 38.0 per cent (0 months) to 94.5 and 91.5 per cent after eight months of storage in CO 2 and Kerala local respectively (Figure 10b). The continued development of mature embryos and development of desiccation tolerance during post harvest ripening might be the probable reason for better germination during these period of storage. The very low germination from the fresh fruit might be due to the dormancy associated with fresh seeds. These results are corroborated with the reports by Saleh Ahmed *et al.* (1987) and Nerson (1991) in cucumber. Maturation drying leads to major developmental changes in seeds of orthodox species (Kermode *et al.*, 1986).

The results with respect to speed of germination, shoot length, root length and vigour index were similar to that of germination during the storage. After eight to nine months of storage, there was a slight decrease in germination and vigour, indicating the on set of seed deterioration inside the fruit during storage. Similar findings in reduction of seed quality during storage was reported by Singh and Singh (1987) in summer squash. However, the reduction was gradual and steady after 12 months of

storage, recording a reduction of 15 and 5.4 per cent in CO 2 and Kerala local, respectively.

Initiation of deterioration might be due to the combined effects of high temperature, low fruit water potential, low oxygen and high CO<sub>2</sub> partial pressures as reported by Edelstein *et al.*, (1995) in muskmelon. In this study, deterioration effect was more pronounced in CO 2 than Kerala local. Edelstein *et al.* (1995) observed that certain cultivars of cucurbits may also be more sensitive to low oxygen environment in deterioration of seeds than others.

Though fruit storage is advantageous for higher seed quality, more space is needed to store the fruits. Keeping such large quantity of fruits are not economically viable. Moreover, during the course of our study rat menace was experienced which is difficult to control and the fruits damaged in the field were vulnerable to fungal infection, which predispose rat menace in the storage room. To achieve complete control, utmost care should be taken for rat control, which involves high cost also. Dormancy of fresh seeds can be overcome by adopting proper fermentation and cleaning methods to get high germination. Therefore, it is suggested that mature fruits should be harvested, extracted and subjected to proper cleaning methods to avoid overhead charges during storage, which will reduce the cost seed production. During unavoidable circumstances, fruit storage is recommended for post harvest maturation and to achieve higher germination. Keeping fruits under storage beyond twelve months may not give benevolent effect on seed quality. If deterioration started inside the fruit, the entire lot will be wasted quickly.

# 5.5. STANDARDISATION OF A SUITABLE METHOD OF SEED EXTRACTION, SEPARATION, WASHING AND DRYING

The process of seed extraction in cucurbits may look easy, but it is crucial as it greatly influence seed quality, particularly the storability. Adoption of faulty extraction method results in counterproductive, instead, simple washing is preferable over time consuming complex procedures (Nerson, 1991). Mechanical and chemical methods of extraction reported to cause seed damage and discolouration respectively, which make seed unfit for storage and sowing because of poor seed quality. Seed extraction by HCl @ 1% of the weight of pulp for 30 minutes found satisfactory and

gave high germination as per the report of Bejoy Kanneth and Devdas (1997) in ash gourd. This is not affordable because of health hazard and faulty use of this method may cause injury and affect the seed quality.

Keeping all the above points carefully, fermentation method is still suitable method for seed extraction in cucurbits. But in ash gourd, proper attention on seed extraction has not been followed by the seed producers and growers. Still, farmers are practicing crude method of extraction i.e. scooping seeds from the pulp and subject them to dry under direct sun light. Sometimes, farmers apply ash to the manually extracted seeds instead of washing before sun drying. This crude method may not remove mucilaginous substances adhered to the seed surface uniformly, thereby invite fungal attack during storage and thus longevity is reduced alarmingly over the period of storage (Bejoy Kenneth and Devdas, 1997). Open fermentation was risky, which invite fungal infection which was confirmed during the course of our study (Plate 14). Moreover, prolonged open fermentation makes pitiful smell and invites maggots development in the fermenting material as well as fungal infection penetrates into the seed coat and cause discolouration. Discolouration can not be removed either by washing or acid. Hence, modification of existing fermentation was thought off. Thus, closed fermentation was evolved to ensure complete removal of mucilaginous substances at the same time avoiding fungal infection, which normally infects from open air.

Fermentation method of extraction was deliberately selected for modification, as any other complex procedure may yield successful result, but may not reach end users, who are ultimately going to adopt the technology. In the present study fermentation (both closed and open) established significant lead over non-fermented seed lot for all the seed quality parameters studied. It is in agreement with Bejoy Kennath and Devdass (1996) who obtained best germination and speed of germination in ash gourd by mere fermentation over non-fermentation in the cultivar BH 21. However, he has not standardised washing and drying steps after fermentation.

Fermentation of seeds along with pulp filled in 300 gauge closed polythene bags and kept for fermentation in dark room (approximately 25°C temperature for

72 h) recorded high germination followed by open fermentation for 72 h (25°C temperature) and non-fermentation methods. The beneficial effect of closed fermentation was pronounced clearly when fermentation was done followed by combination of washing for 15 min., drying in room temperature (25°C) and sun drying (27-30°C) for 12 h. These treatments had independent beneficial effect even without storage. Fruit storage had helped in easy seed extraction and had significant impact on germination to some extent only. Increased germinability due to washing was explained in several cucurbits with respect to leaching of inhibitors by Nerson *et al.* (1985). Hence, all the treatments which had washing as a components showed increased germinability. The improvement of germination by pre-extraction storage in caused by the continuation of embryo development in the harvested fruit.

Since seeds of cucurbit contain germination inhibitors 'cucurbitacins,' germination of seeds are retarded initially until such substances are degraded or metabolized (Watanabe *et al.*, 1988). Washing component in the washing for 15 min., drying at room temperature (25°C) for 72 h and sun drying (27-30°C) for 12 h treatment might help to degrade inhibitors present in the seed coat. Sun drying did not show any positive impact, whereas drying along with washing steps had a positive impact on seed quality increase in ash gourd. Kermode and Bewley (1985) in castor bean and Rachidian and Le Deunff (1986) in muskmelon, explained the positive effect of drying. Significant variations were observed between closed and open fermentation vs non-fermentation method with regard to speed of germination (Plate 15a) during the initial period of storage. Percentage increase in germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after one month of fruit storage is given in Table 57.

Table 57. Percentage increase of germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after one month of fruit storage

Closed fermentation	% increase over non fermentation after one month storage		% increase over open fermentation after one month storage	
	Germination	Speed of germination	Germination	Speed of germination
Sun drying (SD)	42.0	44.8	20.8	19.0
Dying at room temperature (25° C) for 72h + SD	40.2	47.5	11.7	12.3
Washing for 15min + SD	18.0	37.8	7.8	0
Washing for 15 min. +Drying at room (25° C) temperature for 72h+SD	21.8	31.2	6.0	12.8

This was demonstrated by low speed of germination in non-fermentation combined with sun drying, drying at room temperature (25°C) for 72 h + sun drying; washing 15 min + sun drying and washing 15 min + drying (25°C) at room temperature and sun drying for 12 h. Slightly better result was obtained when non-fermentation was combined with washing 15 min. + drying at room temperature for 72 h + sun drying, but that can not be matched with treatments come under closed and open fermentation methods, which recorded superior performance. These results clearly established the fact that non-removal of mucilaginous substances in non-fermented seed prevented germination and their rate of germination (Plate 15b). It was not possible to remove such substances adhered to the seed by mere washing and drying methods without fermentation. The results pertaining to shoot length, root length, seedling dry weight and vigour index followed the same trend as observed in germination.

The results of the present study showed that fermentation had a positive effect on ash gourd seeds, besides higher germination and vigour (Figure 11a and 11b), it is a easy, no cost and low cost seed extraction and separation technology. Closed fermentation 72 h combined with washing for 15 min, drying at room temperature (25°C) for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth.

As the fruit storage period increased, there was no wide difference between closed fermentation and open fermentation as per cent increase of germination and speed of germination was fluctuating. However, even after five months of storage, closed fermentation outperformed non- fermentation method by recording 10.4 and 23.6 per cent increase in germination and speed of germination, respectively. Percentage increase in germination and speed of germination in closed fermentation combined with different washing and drying over non-fermentation and open fermentation after five month of fruit storage is featured in the Table 58.

Table 58. Percentage increase of germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after five month of fruit storage

	% increase over non		% increase over open	
	fermentation after five		fermentation after five months	
Closed fermentation	months storage		storage	
	Germination	Speed of	Germination	Speed of
	Germination	germination	Germmation	germination
Sun drying (SD)	6.5	27.7	-9.5	-5.1
Dying at room temperature (25°C) for 72h + SD	7.1	28.8	0.7	2.6
Washing 15min + SD	4.6	21.1	-1.8	-2.1
Washing 15 min. + Drying at room temperature for 72h+SD	10.0	23.6	3.3	-4.8

From this study, it is inferred that for immediate seed extraction, we should carefully follow, proper separation, washing for 15 min, drying at room temperature(25°C) for 72 h and sun drying (27-30°C) for 12 h after performing fermentation in closed condition for 72 h. Under unavoidable circumstances, if sufficient open room space is available, mere open fermentation method is sufficient after fruit storage for 4 to 5 months, as it recorded little difference over closed fermentation over the period of storage

This modified method can be adopted for large-scale seed production using large airtight containers. While filling the seed material in the airtight containers, hygenic condition of both containers and seed contents must be ensured. This was successfully demonstrated at the seed producers unit K.R. Rangaraj, S/O, Ranganathan, Kondamuthunur, Aryappampalayam (Post), Sathyamangalam (Taluk), Coimbatore (Dt.), Pin 638 452, Phone: 04285 / 251382 and obtained 305 kg quality seed from 45 tonnes of sorted good fruits harvested from 2 acres of ash gourd cv. CO 2 seed crop.

### 5.6. ON FARM TRIAL FOR TEST VERIFICATION OF THE BEST METHOD OF SEED EXTRACTION, SEPARATION WASHING AND DRYING

Quality and storability of seeds are greatly influenced by the post harvest management and handling technologies. Suitable method of seed extraction and separation needs to be adopted to maximise the yield of quality seed in ash gourd. Generally, careful attention is not being given for ash gourd seed extraction and conventional method of extraction affects the seed quality and storability.

It is inferred from the laboratory investigation that closed fermentation for 72 h combined with washing in running water for 15 min. following drying under room temperature (25°C) for 72 h and sun drying for 12 h was the best seed extraction and separation method in ash gourd, by recording higher germination and vigour when compared to open and non fermentation, methods.

In an attempt to make the result to move from lab to land the best method of seed extraction was test verified at seed producers unit (Mr. K.R. Ranagaraj, Kondamuthunur, Sathyamangalam taluk, Erode district, PIN-638452), which yielded good quality seeds of 305 kg from 45 tonnes of sorted good fruits harvested from two acres of CO 2 ash gourd seed crop. The per cent seed recovery was 6.8. Step by step process to be followed for closed fermentation technique in ash gourd is presented in the Flow Chart I. The step by step seed extraction method executed in the seed producers unit is presented in the Plate 16 (a to i).

The cost of extraction for one kilogram of seeds by this closed fermentation method (modified method) was Rs. 56/- which was Rs. 16/- higher than the conventional method. The total cost of cultivation was Rs. 37,275/- for two acres and the Gross income was Rs. 1,52,200/- for two acres. The net income was Rs. 1,14,925/- for two acres (Rs. 57,462.50 / acre) with the cost benefit ratio of 1:3.1 (Table 59 and 60).

In the conventional method, there are difficulties in handling the fruit pulp juice, which causes irritation and allergic to the workers. In the present modified method, fruit pulp and juice handling is minimum and the fermentation is proper in the closed condition. Washing in running water ensures complete removal of mucilaginous substances. The inhibitory substances 'cucurbitacin' which is responsible for dormancy in the fresh seeds are completely degraded and dissolved during the process of closed fermentation, washing and drying.

### CHAPTER V DISCUSSION

"Seed is the quintessential symbol of our self-reliance and the legacy of our agricultural heritage. The changes in weather condition have adverse effect on seed quality and thus, have important consequences for the role of plants in bio-regenerative life support systems, as well as implications for dynamics within the microenvironment of the developing seed" (Musgrave, 2002).

In our condition, manipulation in weather condition is difficult instead, we are contemplating on factors like time of sowing, stage of harvest and post harvest handling to achieve production of quality seeds. In this direction, experiments were conducted in ash gourd varieties, CO 2 and Kerala local to standardize some of the practical seed production aspects related to flowering behaviour as influenced by varieties and seasons, changes during fruit and seed development and maturation, and position of fruit and seed, fruit storage and seed extraction methods on seed quality, at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. On Farm Trial for test verification of the best method of seed extraction, separation, washing and drying was conducted at seed producers unit, Kondamuthunur, Sathyamangalam taluk of Erode district. The results obtained from the experiments are discussed in this chapter.

#### 5.1. FLOWERING BEHAVIOUR AS INFLUENCED BY VARIETIES AND SEASON

Significant influence of season on flowering behaviour was observed in ash gourd and it was also affected by variety. The delay in flower initiation, anthesis, wider sex ratio were the disadvantageous aspects faced by crop raised in summer. In contrast, rainy crop had all the advantages, because of prevailing suitable weather conditions especially adequate rainfall (471 mm), rainy days (26), average temperature (23.8 – 27.5°C), RH (84 to 92%) and solar radiation (350 to 424 ca cm²/d), which culminated in the production of more number of fruits per vine. Weather data recorded during the study period are presented in the Figure 3a, 3b, 3c and 3d.

In both the seasons, the superiority of variety CO 2 over Kerala local was clearly evidenced in the flowering behaviour, except number of fruits per vine. Kamalanathan *et al.* (1972) reported that short day condition with 29.4-29.9°C favours femaleness in ash gourd under Coimbatore condition. More and Munger (1987) observed narrower and wider sex ratio in the rainy and summer crops of muskmelon, respectively. Likwise, Desai and Patil (1985) obtained lower male to female ratio in watermelon variety, Sugar Baby raised in rainy crop (July) than a crop sown on January month.

The present experimental results indicated that inspite of adverse weather condition of high temperature (24.7 to 30.2°C), and inadequate rainfall (108 mm), ash gourd could be established successfully and produced satisfactory number of fruits during summer season. This has proved the characteristics adaptability of ash gourd. But their growth rates are significantly altered due to the season (Nath and Vashistha, 1970). According to Kamalanathan and Thamburaj (1970), pre-flowering and flowering phases in *Cucurbita moschata* were governed mainly by photoperiod and temperature and not affected by humidity and rainfall. But in the present experimental field, rainfall during March, 2002 (69.2 mm) actually boosted the growth of the plants during summer season and that has helped for successful crop.

The present investigation clearly indicated that sowing during the month of July (rainy season) were the best by recording low sex ratio of male to female flowers with maximum numbers of fruits per vine in ash gourd.

# 5.2. FRUIT AND SEED DEVELOPMENT AND MATURATION AS INFLUENCED BY VARIETIES AND SEASONS

In fleshy fruited crops, since seeds are developing inside the fruit, maturity status of both fruits and seeds must be taken into account to obtain maximum quality of seed with full mass maturity, indicated by maximum dry weight, germination and vigour.

To ascertain the correct stages of fruit maturity for maximizing the yield of high quality seed, development and maturation of fruits and seeds were studied in ash gourd varieties CO 2 and Kerala local by growing them in rainy and summer seasons.

Fruit weight, circumference, length, diameter and pulp thickness of ash gourd were found to increase from anthesis to maturity. Regarding fruit weight, it was maximum at 60 DAA in summer season and 70 DAA in rainy season.

During initial growth stages, differences among varieties and influence of season were quite low for the fruit characteristics. The summer crop of both CO 2 and Kerala local produced fruits with smaller diameter when compared to rainy season. The differences in fruit diameter observed between summer and rainy season was 3.5 cm in CO 2 and 1.7 cm in Kerala local at 60 DAA.

Most striking varietal difference observed between CO 2 and Kerala local was the seed cavity. The former had lower diameter with a range from 8.5. to 8.6 cm with large pulp thickness (15.7 to 16.5 cm) which the later possessed higher diameter of seed cavity (11.7 to 11.9 cm) and lower pulp thickness (7.6 - 7.7 cm) during the mature stages of 60-70 DAA.

Good seed recovery was obtained during the later stages of seed development in both seasons and varieties after mass maturity of 55 DAA onwards. Rainy season had 1.18 per cent and summer season recorded more or less equal value of 0.97 per cent as number of filled good seed fraction was high during these stages. Not much variation was observed for fruit characteristics *viz.*, seed cavity and pulp diameter between the seasons.

The variety CO 2 was characterized by producing as high as 9.0 kg of fruit weight and Kerala local found to produce medium size fruits (5.0 kg) with proportionate in the values of other parameters. In rainy season, fruit continues to grow actively as long as it is attached with vine (80 DAA). In summer, the fruit size and weight was culminated much early in 60 DAA and then declined slightly. Balakrishnan *et al.* (1986) obtained increased growth in fruit characteristics as the age of fruits advanced in fleshy fruited papaya. Maximum fruit weight, length and number of seeds per fruit were obtained at 18 and 24 DAA in okra by Somnotra *et al.* (2002) in spring-summer and rainy season, respectively.

With respect to seed, the maximum dry mass accumulation occurred much earlier than fruit (55 DAA) in both seasons. This is in agreement with the findings of Berry and Bewley (1991) in tomato and Jing *et al.* (2000) in and cucumber seeds who reported a poor relationship between fruit and seed development. The reason attributed for continuous weight increase in fruit was moisture accumulation in the fruit pulp, which was restricted in seed beyond its maximum accumulation of dry weight. The amount of water taken by the seed is also a good indicator of seed maturation and germinability and immature seeds take considerably more water than mature seeds (Nerson and Paris, 1988).

Except seed moisture content, all the seed parameters had an increasing trend as age of the ash gourd fruit increased. The maximum fresh and dry weight (mass filling stage) occurred at 55 DAA in summer and it was extended upto 60 DAA in rainy season. In subsequent stages of development and maturation, there was no much change occurred in rainy season for weight of both fresh and dry seed, whereas in summer season drastic reduction of fresh weight was observed (Figure 4a and 4b).

Accumulation of fresh and dry weight of seed was more in Kerala local than CO 2 in all the stages. The varieties CO 2 and Kerala local had accumulated 60 and 72 per cent of drymatter from 20 DAA to mass filling stage in summer season. Whereas, in rainy season both varieties had the highest values of 32 and 60 per cent respectively. These results in ash gourd are tallying with the suggestion of Ellis and Pieta Filho (1992), who described the turning point of maximum dry weight as mass maturity, which was called previously as physiological maturity. Because seed at this point though accumulated maximum dry weight, they are not mature enough to give potential longevity, germination and germination rate. Dry mass accumulation has substantially occurred in developing cotyledon, which is reflected of the measured increase in the cotyledon weight and thickness as reported by Jacks (1990) in cucurbit seeds. Beyond mass maturity, cotyledon had slight increase in accumulation which is negligible when compared to initial rate of thickness and weight increase during mass filling.

The loss of moisture content of seed with time was linear over the range from 59.0 to 43.0 per cent in summer season and 70 to 49 per cent in rainy season during

initial, 20 DAA to mass filling stage, respectively (Figure 4a and 4b). When compared to other cereal crops, reduction in moisture content was not substantial over the period of time as seeds mature and remained within a relatively moist environment in fleshy fruit, where desiccation is prevented or delayed (Welbaum, 1999). In tomato and pepper, the moisture content of mature seeds is maintained at a comparatively high value within the moist fruits. This is more or less similar to all fleshy-fruited crops (Demir and Ellis, 1992a and b). Seed moisture content at maturity was 46 per cent in marrow and it was stabilized to 40 per cent from about 65 DAA onwards (Demir and Ellis, 1993). In this experiment, summer and rainy season fruits had 43-44 per cent and 49-50 per cent of moisture content during the mature stages, respectively.

Germination was initiated in ash gourd at 30 DAA when it was raised in summer, which was approximately 30 days before mass maturity. Semi permeable characteristics of 'cellose', a single layered suberised cover deposited on the outer surface of the endosperm exert resistance to on set of germination during the earlier phase of seed development, which was reported by Yim and Bradford (1998) in muskmelon which is present in ash gourd seed also. Proportion of seeds showing a positive staining in tetrazolium viability test was zero at 30 DAA and increased rapidly to 97.3 per cent in rainy season at 80 DAA and 88.5 per cent in summer season at 65 DAA. This is exactly in agreement with the results obtained in marrow during seed development and maturation by Demir and Ellis (1993). In rainy season, seeds from stage 30 DAA has failed to initiate germination. Muskmelon embryo develop the ability to germinate sequentially well before the accumulation of maximum dry weight, which occurs roughly 35 DAA (Welbaum, 1999).

At 35-55 DAA, moderately mature fruits of ash gourd may disguise the harvester to make mistake for mature one, since entire fruit rind at this stage was covered with ash coat. Hence, once over harvest consisting of mature and immature fruit results in fall of total germination. Seed lots of this kind may not pass minimum seed certification standard (MSCS) of 60 per cent germination in ash gourd. This is the exact reason why there are frequent complaints from seed certification and seed testing authories that seed samples of most of gourds are often failed to pass the minimum seed certification standard for germination.

In summer season, germination initiated from 40 DAA onwards and reached the highest, after mass filling stage and continued to increase till 65 DAA. Rainy season crop maintained the high level of germination upto 80 DAA, whereas in summer it was maintained upto 70 DAA only. At 60 DAA, both CO 2 and Kerala local surpassed the maximum certification standard of 60 per cent germination, irrespective of growing season. During this stage pedicel, of the fruit became dark green, angular, ash coat became powdery and peduncular shoulder depressed to the maximum. The higher germination percentage, vigour index and optimum seed moisture were observed at 27 and 36 days after anthesis in spring summer and rainy season crops, respectively (Saminotra *et al.*, 2002)

There was a little variation occurred among two varieties at this stage (60 DAA) and differences were nullified in subsequent stages for germination. So, harvest may be postponed until last maturity beyond 80 DAA in rainy season and 70 DAA in summerseason. Delay in seed harvesting did not result in substantial decline in potential longevity (Demir and Ellis, 1992) in tomato Demir and Ellis (1993) also observed increased potential longevity, germination and germination rate beyond maximum dry weight accumulation in vegetable marrow.

Ash gourd seeds maintained a near maximum or normal germination for 20 days in summer and 30 days in rainy season from 50 DAA to 70 DAA and 80 DAA respectively. During this period, seed moisture content within the fruit was maintained at values close to 50 per cent which was much higher than the values recorded in mature cereal seeds such as barley (Ellis and Pieta Filho, 1992) and pearlmillet (Kameswara Rao *et al.*, 1991). In fleshy fruited crops, maturation occurs normally after the end of natural seed filling phase and during the pre-harvest maturity period seed deterioration is prevented within the fleshy fruits due to cellular repair and turn-over process. This was reported in tomato by Demir and Ellis (1992) and Kwon and Bradford (1987).

Villiers and Edgecumbe (1975) had proposed the hypothesis that seeds held at moisture content close to full imbibition were provided with free oxygen, but germination was prevented and seeds able to maintain their viability for considerable periods. They postulated that this was because of cellular repair and turn over

activities occur within the seeds, under such conditions, such that any damage that might otherwise accumulate during storage is rapidly repaired. Members of cucurbit crop avoid germination inside the fruit for long time due to the accumulation of osmotic solutes, which creates low water potential inside the developing fruit that maintains seed water potential below the threshold required for germination (Welbaum *et al.*, 1990)

Thus, Villiers hypothesis can also be applied to pre-harvest storage of ash gourd in order to explain the maintenance in maternal environment for 20 to 30 days to get high quality seeds even after achieving mass maturity at 55-60 DAA.

Seed moisture content in the developing and mature fruits of ash gourd remains high throughout the development and maturation. So in fleshy fruited crops, maximum germination was achieved several days after mass maturity as reported in many crops. In contrary, Doijode (1983) and Doijode (1988) obtained maximum germination some days before mass maturity or just after mass maturity in tomato and chilli.

With respect to laboratory germination test, maximum shoot length occurred at 5 and 15 days after mass filling stage in summer and rainy seasons, respectively which coincide with 60 DAA in summer and 70 DAA in rainy season. Similar results were also obtained in case of seed growth and seedling dry weight in tomato by Argerich and Bradford (1989) and Ellis (1989) in onion, respectively.

In the present investigation, a positive correlation was obtained between seed maturity, and germination, shoot and root length, seedling dry weight and vigour index. Fruits harvested beyond 60 and 70 DAA in summer and rainy season respectively yielded quality seeds that showed good strength of physiological parameters *viz.*, germination, shoot and root length, seedling dry weight and vigour index (Plate 9a and 9b). Similar pattern of seed development and maturation was also obtained by Krishna Prasad (1980) in ash gourd cv. CO 1, Bejoy Kennath and Devdass (1996) in BH 21 ash gourd, Hedayat (1987) in watermelon and Adirai (1999) in pumkin.

To conclude this study, seed quality in ash gourd remained high after mass maturity (55-60 DAA) and attained maximum quality which continued upto 70 DAA in summer and 80 DAA in rainy season. In practice, seed producers do not leave fruits on the plant for an expanded period (70-80 DAA) because of the likelihood of viviparous germination and disease or damage by pest. From the results of physiological evaluation, it would appear sensible to suggest that fruits can be harvested 60 DAA in summer season, when pedicle become dried or angular, and 70 DAA in rainy season, when fruits become thick with powdery ash and peduncular end of the fruit shoulder depressed fully. Leaving fruits beyond 80 DAA in rainy season invites fungal growth. During the final maturity stages, seed coat became dark brown detached from the placenta, easily comes out from the pulp when cut open and scooped (Figure 6 and 7). At 70 DAA, almost all the fruit pedicel portion of summer crop became dried (Plate 10a and 10b). Complete crop senescence occurred 170 days after sowing in summer, whereas in rainy season vine and fruit attachment with plants existed beyond 180 days.

Important biochemical changes during seed development was estimated for different stages of seeds from 30 DAA to penultmate maturity. Overall results obtained proved that seed maturity starts from 60 DAA in summer and 70 DAA in rainy season. The measured increase in the thickness of the embryo reflects an increase in the storage reserves especially protein and lipid as reported by Jacks (1990) in cucurbits. Significant difference was not obtained for dehydrogenase enzyme activity and oil content of the seed in the present study. However, numerical increase in values was obtained in the mature stages over immature seeds. The accumulation of protein followed more or less sigmoidal pattern in ash gourd seed, which started from 30 DAA and continued upto 70 DAA in summer season while rainy crop had protein accumulation beyond 70 DAA and continued upto 80 DAA. However, after 50 DAA, accumulation was stablised. Similar pattern of protein and oil accumulation was recorded in developing seeds of brassica by Dasgupta and Mandal (1993).

#### **Histological studies**

#### Seed coat development

Structural changes in seed coat during seed development and maturation examined using microtome sectioning are presented in Plates 11a to 11c.

According to Dathan and Singh (1990) seed coat development in cucurbit is peculiar during development and maturation of seed. It was evident from the microtome sections that structural changes in seed coat of mature seeds were entirely different from the seed coat of immature seeds. The seed coat of over mature seed was visible as non-structured cell walls and at the peak maturity stage, multiple layers of cell wall was visible with maximum expansion. Elongated tubular or rod shaped thickening in epidermal cell running in the radial direction from the inside to outside, was visible in the seed coat of mature seeds of ash gourd, which was absent in the seed coat of immature seeds. The same structure with different shape was observed in Cucurbita pepo by Singh (1953). In immature seed, seed coat layers were so compact that it was difficult to split by growing embryo. Similar finding was expressed by Edelstein et al. (1995) and Bewley and Black (1982) in muskmelon, and they opined that seed coat and other surrounding structures may influence germination by exerting mechanical restriction to embryo growth. In seeds of melon, the embryo was enclosed by a testa and a thin perisperm, that were permeable to water (Welbaum and Braford, 1990). In the present study, the testa got thickened as age of seed increased and it was stopped ahead of cotyledon expansion.

#### Embryo development

The structural changes in embryo during the seed development and maturation through microtome examination are depicted in the Plates 12a (i to vii).

Like most dicotyledonous species (Raven *et al.*, 1992), embryo morphology in ash gourd seed during embryogenesis proceed through pre-embryo and globular at early cotyledon stages and enlargement in the middle stages (30-50 DAA) and filling and maturation in the late stages (60-80 DAA). Ma and Zheng (1992) have classified into stages of the embryo development in Chinese cabbage seeds according to its

morphology and they recorded embryo development from 5 to 10 DAA as a single stage. Kuang *et al.* (1996) divided *Arabidopsis thaliana* embryo development into six stages as late globular, cotyledon initiation, early linear cotyledon stage, late linear cotyledon stage, cotyledon stage and mature embryo.

In general, seed development was divided into embryo cell division and differentiation, rapid accumulation of storage materials and dehydration as per the findings of Dasgupta and Mandal (1990) observed in *Brassica* seed. Accordingly, in ash gourd, maximum expansion of cells was observed in last maturation stage (60 DAA) which came under second phase as per dicotyledenary seed development. In ash gourd, this second phase was either delayed or extended even after attaining full maturity. During the intermediate development stages (30-50 DAA), rapid accumulation of storage materials was observed. During the initial development of cotyledon (20-25 DAA), cell division was completed which was evident from the sections taken from the developing, immature and young seeds (20-30 DAA). Aaccording to Yim and Bradford (1998), muskmelon seed also stopped their cell division during 30-35 DAA.

In the present study, the cotyledons developed the ability to expand first (20 DAA), followed by embryonic axis development (30-60 DAA). During embryonic axis development, hypocotyls expanded first, followed by root and shoot meristems. This is similar to the results observed in muskmelon seeds by Welbaum and Bradford (1989).

In this present study, late globular stage occurred approximately at 5 DAA. Early stage of cotyledenary development was observed at 15 DAA. At 20 DAA, complete cotyledenary structure had developed. During the subsequent stages (upto 50 DAA), cotyledon thickening and embryo differentiation took place. Mature embryo could be observed beyond 50-60 DAA. From over mature seed stage (80 DAA), radicle cells (Plate 12b (i to vii) were highly enlarged and multiple growth

was observed in hypocotyl axis. Cell enlargement in all other stages was proportionate to their increased growth stages.

#### Measurement of seed and embryo descriptors using image analysis system

The developing and maturing seeds and embryo were subjected to image analysis systems for observing the variation in the physical characteristics.

This method had broad potential usefulness as a rapid indicator of seed lot quality. An appropriate integration between automated machine vision techniques and standard seed evaluation tests could provide more detailed information on the status of seed lot (Heijden *et al.*, 1999). The image analysis method was used to measure ash gourd seed size and shape based on perimeter, area, elongation, circularity, length, width and shape factor. All the parameters were found useful in fixing the pattern of the maturity status of seed. The data obtained were also used to findout the difference in the distinguishable seed morphological characteristics of CO 2 from Kerala local (Figure 8a and 8b). According to Vooren *et al.* (1991), crop varieties can be identified based on shape descriptors like area, perimeter and shape factor. They have identified the parameters showing significant differences. According to Drzewiecki *et al.* (2000), size and shape of the seeds are typical of a species and variety.

In rainy season crop the study of seed characteristics of both the varieties using image analysis system showed that area, perimeter, length and width of the cotyledon as well as continuity of seed growth reached the highest values at 70-80 DAA, which was coincided with the stages after mass filling maturity stage. Most significant point observed here was that there existed of a varietal variation, between the two varieties for all the stages studied starting from 20 DAA to 80 DAA.

The variety Kerala local had registered higher geometric values listed above, indicating bigger size seeds, when compared to CO 2. Except numerical difference,

cotyledon and embryo showed the similar trend with respect to growth and development. Very high variation was observed with regard to width of seed and cotyledon. Correct and accurate varietal identification was possible based on width or shape factor as suggested by Drzewiecki *et al.* (2000). Many seeds are similar in shape, but differ widely in size. Size is often used in description of plant seeds in order to assess identification (Shieh and McDonald, 1982). Significant finding observed here were the elongation and circularity values of cotyledon and seeds, which were more in the initial growth stages (30 DAA) and as the growth increased, the valves were also stabilised. All the measured valves of seed shape factor exceeded 1.0, which is the maximum theoretical value expected. It is a feature of the picture element and does not affect the utility of data. At initial stages (30-40 DAA) of seed development and maturation, shape factor value was higher than mature (50-70 DAA) stages.

The conclusion from this study is that by using machine system a good varietal discrimination could be established and growth of objects could be monitored for maturity status of the seed during development and maturation. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local confirmed their maturity stages, as both cotyledon and seeds of mature stages observed to have more values of seed descriptors. According to Edwards and Lowe (1981), image analysis method can be used apart from capturing binary images and subsequent measurements of size and shape in relation to germination and growth performance in laboratory and field.

Positive relationship was observed for growth stages with seed descriptor values and seed performance in terms of germination. Seed descriptors using image analysis system could be done very quickly and large volume of samples could be subjected to observation within a shorter period of time.

#### Gel electrophoresis of total protein

Gel electrophoresis of protein at different stages of ash gourd seed development revealed that occurrence of mature and processed storage proteins in the bands representing seed stages of 40 to 70 and 80 DAA in summer and rainy season crops, respectively. In the initial stages (20-30 DAA), very less accumulation with lower contractions was noticed. High molecular weight proteins were totally absent in both varieties of ash gourd, which could be observed in all the lanes of both seasons (Plate 13). Pasha (1986) and Pasha and Sen (1991) have examined the electrophoretic patterns of seed protein in mature seeds of cucurbits and detected 3-5 bands having moderate molecular weights, which contributed major fractions.

In the present study, identical banding pattern was reflected in both summer and rainy seasons, but varietal variation observed as Kerala local showed extra banding especially in the less molecular protein area (lower zones among four zones) in 20 and 14.3 Kda. Expression was high at 20 Kda than 14.3 kda. Pasha and Sen (1989) reported difference of protein band having lower molecular weight in *Cucumis melo* var. *melo* and var. *agrestis*. The major reserve seed proteins reported in cucurbits seeds was 11S globulin (Guha and Sen, 1998) which was expressed in this study at 20 kda and adjoining flow area 2S globulin was expressed in 14.3 kda.

Crouch and Sussex (1981) found that onset of storage protein accumulation in *Brassica* sp. coincided with the beginning of phase two of embryo growth which was roughly 30 DAA in ash gourd as defined in histological studies. In contrary, Norton and Harris (1975) showed that major proteins found in the mature seeds of *Brassica* do not appear in the initial stages of seed development. This is in agreement with the results obtained in this study, as initial stages of ash gourd showed very less protein concentrations in the form of bands. During the first phase of cotyledon development, cotyledon was small part of the seed and its proteins would thus be diluted (Norton *et al.*, 1976). Since, the whole seeds were used for gel electrophoresis, very

light concentrations of bands were obtained in this study. Norton *et al.* (1976) also expressed similar view.

From the electrophoresis study, it is concluded that precise maturity detection is not possible as mature processed proteins exhibit same bands all along starting from 40 to 80 DAA in rainy season, and 4 to 70 DAA in summer season. As per the physio-biochemical studies, stages ranged between 40-50 DAA were moderately mature and putforth very less germination. There was no striking difference with respect to banding pattern between fully mature seeds (60-80 DAA) and moderately mature seeds (40-50 DAA). However, by and large immature stages (20-30 DAA) could be very well differentiated from that of moderately mature to mature stages. This study is very much useful for varietal identification and helpful as basic information for molecular level investigations in ash gourd in future

#### 5.3. INFLUENCE OF POSITION OF FRUIT AND SEED ON SEED QUALITY

There was a significant variation for seed moisture content, seed dry weight, germination, field emergence and seedling dry weight due to position of fruits and seed. At peak maturity of 70 DAA, bottom and top fruits had more or less equal seed moisture content (30.9 and 30.8 per cent), whereas middle fruit had much higher (39.5 per cent) moisture content. Peduncular segment in all the fruit position showed higher moisture content in all the stages (Figure 9a). Such difference in moisture content was also observed in marrow, which occurred 65 DAA, as per the report by Demir and Ellis (1993).

In ash gourd, dry weight of seed increased steadily and reached a maximum at 70 DAA except for peduncular segments, in which the increase in seed dry weight was not encouraging which recorded low dry weight in all the stages. Same differences continued upto 70 DAA with little variation than earlier stages. During the initial stages (30 DAA), basal fruit was the best, recording higher seed dry weight compared to middle and top fruits. But as the maturity stages increased, top fruits had more seed dry weight than remaining position.

Khan and Passom (1992) obtained higher fruit weight and lower seed content in bottom fruit of sweet pepper. Similar increase in seed weight of top fruits was obtained by Jambhale et al. (1988). Thus, it may be worthwhile to harvest fruits from top of the vine for obtaining higher seed recovery. Among different segments, peduncular had very low seed weight. Severe competition occurs among reproductive sinks, maternal resources whose relative developmental stages and position on mother plant influence seed performance (Watson and Casper, 1984; Rocha and Stephens, 1991). The results confirmed the variation in seed dry weight, germination and emergence as observed in the present study. Ash gourd seeds like other cucurbits, acquired germinability prior to the attainment of mass maturity and initiation of seed germination started at 30 DAA from top positioned fruits. This has confirmed the previous findings (Nerson and Paris, 1988) that the process of germination does not require completion of seed development. After 40 DAA, seeds from three positions showed differences in the accumulation of dry weight and germination percentage. Hence, there was a difference in seed quality in the early maturity stage prior to mass maturity, which was revealed by germination and field emergence Nerson and Paris (1988) had a opinion that for initiation of germination, cucurbit seed does not need full seed development. Seed quality variation due to position is in agreement with the performance of tomato and muskmelon seeds observed by Welbaum and Bradford (1989), Berry and Bewley (1991) and Demir and Ellis (1992).

In cucumber also, similar results were reported by Nerson (1991). Seed from various position achieved maximum germination and produced normal seedlings, but showed different values. Seeds from peduncular segments of bottom, middle and top produced low germination. In ash gourd, seeds closer to the stem exhibited lower performance. Seeds from top fruits outperformed middle and bottom fruits (Figure 9b). The mature seeds from top fruits was heavier than those from middle and bottom. This is in conformity with the result of tomato reported by Demir and Ellis (1992), who stated that seeds from the upper truss exhibited good performance than seeds from the lower truss.

The observed lower seed quality within the position might be due to dominance effects of top fruits over the others (Marcelis, 1993). The poor performance from the peduncular segment of a fruit might be a result of fertilization

with slow germinating pollen, which might in turn show low germination and vigour than that of fertilizing ovules at the stylar end of the overy (Lau and Stephenson, 1993). In contrary, Doijode (1990) and Doijode (2000) obtained best quality seeds from peduncular segment of the chilli and brinjal fruits. This variation might be due to the pollination behaviour as these crops are self pollinated and fertilization effect might not influence seed performance like in ash gourd, which is a highly cross pollinated crop.

In ash gourd, variation due to position of fruits and segments exhibited more during the early stages, it prolonged upto 60 DAA. After attaining 70 DAA, variation in seed quality was less. Overall results revealed that performance of seeds from 70 DAA was excellent when compared to other stages. Hence, prolonged maturation of seeds inside the fruit is beneficial for seed quality point of view. This study also confirmed that harvesting of fruits at 70 DAA is a correct and suitable stage for best seed quality in rainy season crop.

From this study it is very clear that in species with linear arrangement of seeds within the fruits, seed position may influence seed quality, which is affected by gametophytic selection. Ovules present in the peduncular segment had lesser opportunity for fertilization than stylar segment, that is why the performance of seeds from peduncular portion is less. In predominantly cross pollinated crop having linear seed arrangement, the influence gets multiplied.

Fruits borne in the early nodes may have advantage of better fruit growth and seed weight increase in the initial stages, as growth of plants progress, the source sink resource will be diverted to seed and in many of the large fruited crops low seed weight was reported. In the apical portion of the plants, fruit size may increase or decrease, but seed content will be better, consequently more seed recovery also. From this study, it is concluded that the ash gourd fruits from the top nodes (beyond 47<sup>th</sup> node) could be utilised for seed purpose and from the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised for vegetable purpose.

#### 5.4. EFFECT OF FRUIT STORAGE ON SEED QUALITY

It is a general practice that gourd fruits are harvested at vegetable maturity and stored for seed extraction in the farmers holding. But leaving the fruits in the vine for full maturity is more effective according to Nerson (1991). Besides the initial germination, longevity of such seeds harvested at the time of vegetable maturity is also poorer than those mature on the mother plant itself. In practice, seed growers may not take risk in retaining fruits in the field. In this context, storage of fruits is highly essential for quality seed production point of view.

Pre-extraction storage of harvested fruits improves germinability in many cucurbits (Wallerstein *et al.*, 1981; Alvernga *et al.*, 1984). Hence, influence of extended storage of mature (70 DAA) ash gourd fruits under room storage condition was studied using CO 2 and Kerala local varieties. During the course of storage, there was continuous weight loss in both the varieties upto 12 months period due to water evaporation, which resulted in dehydration of fruits leaving pithy pulp and seed mass intact. The extend of weight loss was 53 and 49 per cent recorded in CO 2 and Kerala local, respectively (Figure 10a). The moisture content of the seed decreased from 57.9 and 54.4 to 31.0 and 37.0 per cent, respectively. The loss of fresh and dry weight of seed was reported to be 23.4 and 10.2 per cent and 5.3 and 10.8 per cent in CO 2 and Kerala local, respectively. The weight loss of 4 per cent after three weeks of storage was recorded in muskmelon under room condition at 15°C (Miccolis and Saltveit, 1995). They also reported varietal variation in per cent weight loss.

Germination of seeds significantly increased from 36.5 and 38.0 per cent (0 months) to 94.5 and 91.5 per cent after eight months of storage in CO 2 and Kerala local respectively (Figure 10b). The continued development of mature embryos and development of desiccation tolerance during post harvest ripening might be the probable reason for better germination during these period of storage. The very low germination from the fresh fruit might be due to the dormancy associated with fresh seeds. These results are corroborated with the reports by Saleh Ahmed *et al.* (1987) and Nerson (1991) in cucumber. Maturation drying leads to major developmental changes in seeds of orthodox species (Kermode *et al.*, 1986).

The results with respect to speed of germination, shoot length, root length and vigour index were similar to that of germination during the storage. After eight to nine

months of storage, there was a slight decrease in germination and vigour, indicating the on set of seed deterioration inside the fruit during storage. Similar findings in reduction of seed quality during storage was reported by Singh and Singh (1987) in summer squash. However, the reduction was gradual and steady after 12 months of storage, recording a reduction of 15 and 5.4 per cent in CO 2 and Kerala local, respectively.

Initiation of deterioration might be due to the combined effects of high temperature, low fruit water potential, low oxygen and high CO<sub>2</sub> partial pressures as reported by Edelstein *et al.*, (1995) in muskmelon. In this study, deterioration effect was more pronounced in CO 2 than Kerala local. Edelstein *et al.* (1995) observed that certain cultivars of cucurbits may also be more sensitive to low oxygen environment in deterioration of seeds than others.

Though fruit storage is advantageous for higher seed quality, more space is needed to store the fruits. Keeping such large quantity of fruits are not economically viable. Moreover, during the course of our study rat menace was experienced which is difficult to control and the fruits damaged in the field were vulnerable to fungal infection, which predispose rat menace in the storage room. To achieve complete control, utmost care should be taken for rat control, which involves high cost also. Dormancy of fresh seeds can be overcome by adopting proper fermentation and cleaning methods to get high germination. Therefore, it is suggested that mature fruits should be harvested, extracted and subjected to proper cleaning methods to avoid overhead charges during storage, which will reduce the cost seed production. During unavoidable circumstances, fruit storage is recommended for post harvest maturation and to achieve higher germination. Keeping fruits under storage beyond twelve months may not give benevolent effect on seed quality. If deterioration started inside the fruit, the entire lot will be wasted quickly.

# 5.5. STANDARDISATION OF A SUITABLE METHOD OF SEED EXTRACTION, SEPARATION, WASHING AND DRYING

The process of seed extraction in cucurbits may look easy, but it is crucial as it greatly influence seed quality, particularly the storability. Adoption of faulty extraction method results in counterproductive, instead, simple washing is preferable

over time consuming complex procedures (Nerson, 1991). Mechanical and chemical methods of extraction reported to cause seed damage and discolouration respectively, which make seed unfit for storage and sowing because of poor seed quality. Seed extraction by HCl @ 1% of the weight of pulp for 30 minutes found satisfactory and gave high germination as per the report of Bejoy Kanneth and Devdas (1997) in ash gourd. This is not affordable because of health hazard and faulty use of this method may cause injury and affect the seed quality.

Keeping all the above points carefully, fermentation method is still suitable method for seed extraction in cucurbits. But in ash gourd, proper attention on seed extraction has not been followed by the seed producers and growers. Still, farmers are practicing crude method of extraction i.e. scooping seeds from the pulp and subject them to dry under direct sun light. Sometimes, farmers apply ash to the manually extracted seeds instead of washing before sun drying. This crude method may not remove mucilaginous substances adhered to the seed surface uniformly, thereby invite fungal attack during storage and thus longevity is reduced alarmingly over the period of storage (Bejoy Kenneth and Devdas, 1997). Open fermentation was risky, which invite fungal infection which was confirmed during the course of our study (Plate 14). Moreover, prolonged open fermentation makes pitiful smell and invites maggots development in the fermenting material as well as fungal infection penetrates into the seed coat and cause discolouration. Discolouration can not be removed either by washing or acid. Hence, modification of existing fermentation was thought off. Thus, closed fermentation was evolved to ensure complete removal of mucilaginous substances at the same time avoiding fungal infection, which normally infects from open air.

Fermentation method of extraction was deliberately selected for modification, as any other complex procedure may yield successful result, but may not reach end users, who are ultimately going to adopt the technology. In the present study fermentation (both closed and open) established significant lead over non-fermented seed lot for all the seed quality parameters studied. It is in agreement with Bejoy Kennath and Devdass (1996) who obtained best germination and speed of germination in ash gourd by mere fermentation over non-fermentation in the cultivar

BH 21. However, he has not standardised washing and drying steps after fermentation.

Fermentation of seeds along with pulp filled in 300 gauge closed polythene bags and kept for fermentation in dark room (approximately 25°C temperature for 72 h) recorded high germination followed by open fermentation for 72 h (25°C temperature) and non-fermentation methods. The beneficial effect of closed fermentation was pronounced clearly when fermentation was done followed by combination of washing for 15 min., drying in room temperature (25°C) and sun drying (27-30°C) for 12 h. These treatments had independent beneficial effect even without storage. Fruit storage had helped in easy seed extraction and had significant impact on germination to some extent only. Increased germinability due to washing was explained in several cucurbits with respect to leaching of inhibitors by Nerson *et al.* (1985). Hence, all the treatments which had washing as a components showed increased germinability. The improvement of germination by pre-extraction storage in caused by the continuation of embryo development in the harvested fruit.

Since seeds of cucurbit contain germination inhibitors 'cucurbitacins,' germination of seeds are retarded initially until such substances are degraded or metabolized (Watanabe *et al.*, 1988). Washing component in the washing for 15 min., drying at room temperature (25°C) for 72 h and sun drying (27-30°C) for 12 h treatment might help to degrade inhibitors present in the seed coat. Sun drying did not show any positive impact, whereas drying along with washing steps had a positive impact on seed quality increase in ash gourd. Kermode and Bewley (1985) in castor bean and Rachidian and Le Deunff (1986) in muskmelon, explained the positive effect of drying. Significant variations were observed between closed and open fermentation vs non-fermentation method with regard to speed of germination (Plate 15a) during the initial period of storage. Percentage increase in germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after one month of fruit storage is given in Table 57.

Table 57. Percentage increase of germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after one month of fruit storage

Closed fermentation	% increase over non fermentation after one month storage		% increase over open fermentation after one month storage	
	Germination	Speed of germination	Germination	Speed of germination
Sun drying (SD)	42.0	44.8	20.8	19.0
Dying at room temperature (25° C) for 72h + SD	40.2	47.5	11.7	12.3
Washing for 15min + SD	18.0	37.8	7.8	0
Washing for 15 min. +Drying at room (25° C) temperature for 72h+SD	21.8	31.2	6.0	12.8

This was demonstrated by low speed of germination in non-fermentation combined with sun drying, drying at room temperature (25°C) for 72 h + sun drying; washing 15 min + sun drying and washing 15 min + drying (25°C) at room temperature and sun drying for 12 h. Slightly better result was obtained when non-fermentation was combined with washing 15 min. + drying at room temperature for 72 h + sun drying, but that can not be matched with treatments come under closed and open fermentation methods, which recorded superior performance. These results clearly established the fact that non-removal of mucilaginous substances in non-fermented seed prevented germination and their rate of germination (Plate 15b). It was not possible to remove such substances adhered to the seed by mere washing and drying methods without fermentation. The results pertaining to shoot length, root length, seedling dry weight and vigour index followed the same trend as observed in germination.

The results of the present study showed that fermentation had a positive effect on ash gourd seeds, besides higher germination and vigour (Figure 11a and 11b), it is a easy, no cost and low cost seed extraction and separation technology. Closed fermentation 72 h combined with washing for 15 min, drying at room temperature (25°C) for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth.

As the fruit storage period increased, there was no wide difference between closed fermentation and open fermentation as per cent increase of germination and speed of germination was fluctuating. However, even after five months of storage, closed fermentation outperformed non- fermentation method by recording 10.4 and 23.6 per cent increase in germination and speed of germination, respectively. Percentage increase in germination and speed of germination in closed fermentation combined with different washing and drying over non-fermentation and open fermentation after five month of fruit storage is featured in the Table 58.

Table 58. Percentage increase of germination and speed of germination in closed fermentation combined with different washing and drying treatments over non-fermentation and open fermentation after five month of fruit storage

Closed fermentation	% increase over non fermentation after five months storage		% increase over open fermentation after five months storage	
	Germination	Speed of germination	Germination	Speed of germination
Sun drying (SD)	6.5	27.7	-9.5	-5.1
Dying at room temperature (25°C) for 72h + SD	7.1	28.8	0.7	2.6
Washing 15min + SD	4.6	21.1	-1.8	-2.1
Washing 15 min. + Drying at room temperature for 72h+SD	10.0	23.6	3.3	-4.8

From this study, it is inferred that for immediate seed extraction, we should carefully follow, proper separation, washing for 15 min, drying at room temperature(25°C) for 72 h and sun drying (27-30°C) for 12 h after performing fermentation in closed condition for 72 h. Under unavoidable circumstances, if sufficient open room space is available, mere open fermentation method is sufficient after fruit storage for 4 to 5 months, as it recorded little difference over closed fermentation over the period of storage

This modified method can be adopted for large-scale seed production using large airtight containers. While filling the seed material in the airtight containers, hygenic condition of both containers and seed contents must be ensured. This was successfully demonstrated at the seed producers unit K.R. Rangaraj, S/O, Ranganathan, Kondamuthunur, Aryappampalayam (Post), Sathyamangalam (Taluk), Coimbatore (Dt.), Pin 638 452, Phone: 04285 / 251382 and obtained 305 kg

quality seed from 45 tonnes of sorted good fruits harvested from 2 acres of ash gourd cv. CO 2 seed crop.

### 5.6. ON FARM TRIAL FOR TEST VERIFICATION OF THE BEST METHOD OF SEED EXTRACTION, SEPARATION WASHING AND DRYING

Quality and storability of seeds are greatly influenced by the post harvest management and handling technologies. Suitable method of seed extraction and separation needs to be adopted to maximise the yield of quality seed in ash gourd. Generally, careful attention is not being given for ash gourd seed extraction and conventional method of extraction affects the seed quality and storability.

It is inferred from the laboratory investigation that closed fermentation for 72 h combined with washing in running water for 15 min. following drying under room temperature (25°C) for 72 h and sun drying for 12 h was the best seed extraction and separation method in ash gourd, by recording higher germination and vigour when compared to open and non fermentation, methods.

In an attempt to make the result to move from lab to land the best method of seed extraction was test verified at seed producers unit (Mr. K.R. Ranagaraj, Kondamuthunur, Sathyamangalam taluk, Erode district, PIN-638452), which yielded good quality seeds of 305 kg from 45 tonnes of sorted good fruits harvested from two acres of CO 2 ash gourd seed crop. The per cent seed recovery was 6.8. Step by step process to be followed for closed fermentation technique in ash gourd is presented in the Flow Chart I. The step by step seed extraction method executed in the seed producers unit is presented in the Plate 16 (a to i).

The cost of extraction for one kilogram of seeds by this closed fermentation method (modified method) was Rs. 56/- which was Rs. 16/- higher than the conventional method. The total cost of cultivation was Rs. 37,275/- for two acres and the Gross income was Rs. 1,52,200/- for two acres. The net income was Rs. 1,14,925/- for two acres (Rs. 57,462.50 / acre) with the cost benefit ratio of 1:3.1 (Table 59 and 60).

In the conventional method, there are difficulties in handling the fruit pulp juice, which causes irritation and allergic to the workers. In the present modified method, fruit pulp and juice handling is minimum and the fermentation is proper in the closed condition. Washing in running water ensures complete removal of mucilaginous substances. The inhibitory substances 'cucurbitacin' which is responsible for dormancy in the fresh seeds are completely degraded and dissolved during the process of closed fermentation, washing and drying.

### **Abstract**

# Harvesting, post harvest handling and conditioning of fruit and seed in ash gourd (BENINCASA HISPIDA (THUNB.) COGN)

By

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**DEGREE**: **DOCTOR OF PHILOSOPHY (AGRICULTURE)** 

IN SEED SCIENCE AND TECHNOLOGY

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COIMBATORE - 641 003

2003

Experiments were conducted in ash gourd varieties, co 2 and kerala local to document the flowering behaviour and changes during fruit and seed development and maturation as influenced by varieties and seasons; and position of fruit and seed, fruit storage and seed extraction methods on seed quality at the department of seed science and technology, tamil nadu agricultural university, coimbatore. An on farm trial for test verification of the best method of seed extraction, separation, washing and drying was conducted at seed producers unit, kondamuthunur, sathyamangalam taluk of erode district.

Sowing ash gourd during the month of July (rainy season) was observed best by recording low sex ratio and maximum numbers (five) of fruits per vine. Alteration of flowering behaviour was observed to be influenced by seasons. Ash gourd was successfully established with reasonable number (two per vine) of fruits during summer season also, but they showed wider sex ratio.

Germination was initiated in ash gourd at 30 days after anthesis, when it was raised in summer season, which was approximately 30 days before mass maturity. In rainy season, seeds from stage 30 DAA has failed to germinate. In summer season, normal germination (62 per cent) initiated during 55 days after anthesis onwards and reached the highest after mass filling stage and continued to increase till 65 DAA (83 per cent). Rainy crop maintained the high level of germination (84.5 per cent) upto 80 DAA, whereas in summer it was maintained (76.5 per cent) upto 70 DAA only.

Ash gourd seeds maintained, near maximum or normal germination for 20 days in summer and 30 days in rainy season from 50 DAA to 70 DAA and 80 DAA respectively. Fruits harvested beyond 60 and 70 DAA in summer and rainy respectively yielded quality seeds that showed good strength of physiological parameters *viz.* germination, shoot and root length, seedling dry weight and vigour index.

Hence, fruits can be harvested 60 DAA in summer season, when pedicel become dried or angular, and 70 DAA in rainy season, when fruits became thick with powdery ash and peduncular end of the fruit shoulder depressed fully. Leaving fruits beyond 80 DAA in rainy season invite fungal growth. During the final maturity stages, seed coat became dark brown detached from the placenta, easily comes out from the pulp when cut open and scooped. At 70 DAA, almost all the fruit pedicel portion of summer crop became dried. Complete crop senescence occurred 170 days after sowing in summer, whereas in rainy season vine and fruit attachment with plants existed beyond 180 days.

Microtome section examination revealed that elongated tubular or rod shaped thickening in epidermal cells running in the radial direction from the inside to outside, was visible in the seed coat of matured seeds of ash gourd, which was absent in the seed coat of immature seeds. Using image analysis system, good varietal discrimination could be established and growth of objects could be monitored for maturity status of the seed during development and maturation. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local confirmed their

maturity stages, as both cotyledons and seeds of matured stages observed to have more values of seed descriptors.

From the electrophoresis study, it is concluded that precise maturity detection could not possible as matured processed proteins exhibit same bands all along starting from 40 to 80 DAA in rainy season, and 40 to 70 DAA in summer season. High molecular weight proteins were totally absent in both the varieties of ash gourd, which could be observed in all the lanes of both seasons.

Seeds from peduncular segments of bottom, middle and top fruits produced the lowest germination. In ash gourd, seeds closer to the stem exhibited lower performance. Seeds from top fruits outperformed middle and bottom fruits. The mature seeds from top fruits was heavier than those from middle and bottom fruits. From this study, it is concluded that the ash gourd fruits from the top nodes (>46<sup>th</sup> node) could be utilised for seed purpose and from the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised for vegetable purpose.

Germination of seeds significantly increased from 36.5 and 38.0 per cent (at the time of storage) to 94.5 and 91.5 per cent after eight months of storage in CO 2 and Kerala local, respectively. The results with respect to speed of germination, shoot length, root length and vigour index were similar to that of germination during the storage. After eight to nine months of storage, there was a slight decrease in germination and vigour, indicating the on set of seed deterioration inside the fruit during fruit storage. Fruit storage is recommended for post harvest maturation and to achieve higher germination. Keeping fruits under storage beyond twelve months would not give benevolent effect on seed quality.

The results of method of seed extraction showed that closed fermentation had a positive effect on ash gourd seeds, besides higher germination and vigour, it is a easy and low cost technology for seed extraction and separation in ash gourd. Closed fermentation for 72 h combined with washing for 15 min, drying at room temperature (25°C) for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth and non fermentation gave poor germination and vigour.

This modified method of seed extraction was successfully demonstrated at the seed producers field, Mr. K.R.Ranagaraj, Kondamuthunur, Sathyamangalam taluk, Erode district, and obtained 305 kg of good quality seeds from 4.5 tonnes good quality fruits of CO 2 ash gourd harvested from 2 acres of area. To extract one kilogram of seeds, Rs. 56 was incurred due to closed fermentation method in a large scale seed production unit. The cost benefit ratio was 1:3.1.

### **CHAPTER VI**

#### **SUMMARY**

The results of experiments carried out with ash gourd varieties CO 2 and Kerala local on flowering behaviour, fruit and seed development and maturation as influenced by varieties and seasons, influence of fruit and seed position on seed quality, effect of fruit storage on seed quality, standardisation of a suitable method of seed extraction, separation, washing and drying and On Farm Trail on seed extraction are summerised in this chapter.

### 6.1. FLOWERING BEHAVIOUR AS INFLUENCED BY VARIETIES AND SEASONS

Significant influence of season on flowering behaviour was observed in ash gourd and it was also affected by variety. In both the seasons, the superiority of variety CO 2 over Kerala local was clearly brought out with respect to flowering behaviour, except number of fruits per vine. The present experimental result indicated that inspite of adverse weather condition of high temperature (24.7 to 30.2°C) and inadequate rainfall (108 mm), ash gourd could be established successfully and yielded reasonable number of fruits (Two fruits per vine) during summer season.

The present investigation clearly indicated that sowing during the month of July (rainy season) was observed best by recording low sex ratio of male to female flowers with maximum (five) number of fruits per vine in ash gourd. However, seed crop can be grown in both seasons (rainy and summer seasons) and alteration of flowering behaviour especially, sex ratio is unavoidable in summer season.

### 6.2. FRUIT AND SEED DEVELOPMENT AND MATURATION AS INFLUENCED BY VARIETIES AND SEASONS

In fleshy fruited crops, since seeds are developing inside the fruit, maturity status of both fruits and seeds must be taken into account to obtain maximum quality of seed with full mass maturity, indicated by maximum dry weight, germination and vigour. Fruit weight, circumference, length, diameter and pulp thickness of ash gourd were found to increase from anthesis to maturity. Regarding fruit weight, it was maximum at 60 DAA in summer season and 70 DAA in rainy season.

Good seed recovery was obtained during the later stages of seed development in both seasons and varieties after mass maturity of 55 DAA onwards. With respect to seed, maximum dry weight accumulation occurred much earlier than fruit (55 DAA) in both seasons. The maximum fresh and dry weight (mass filling stage) occurred at 55 DAA in summer and it was extended upto 60 DAA in rainy season. Subsequent stages of development and maturation, there was no much change in rainy season for weight of both fresh and dry seed, whereas in summer season drastic reduction of fresh weight was observed. Accumulation of fresh and dry weight of seed was more in Kerala local than CO 2 in all the stages.

The loss of moisture content of seed with time was linear over the range from 59.0 to 43.0 per cent in summer season and 70 to 49 per cent in rainy season during initial and 20 DAA to mass filling stage, respectively.

Germination was initiated in ash gourd at 30 DAA when it was raised in summer, which was approximately 30 days before mass maturity. Semi-permeable characteristics of 'cellose', a single layered suberised cover deposited on the outer surface of the endosperm exert resistant to on set of germination during the earlier phase of seed development. In rainy season, seeds from stage 30 DAA has failed to initiate germination.

In summer season, germination initiated during 40 DAA onwards and reaches the highest after mass filling stage (55 DAA) and continued to increase (83 per cent) till 65 DAA. Rainy crop maintained the high level of germination (84.5 per cent) upto 80 DAA, whereas in summer crop it (76.5 per cent) was maintained upto 70 DAA only. Ash gourd seeds maintained near maximum or normal germination for 20 days in summer season (75.6 per cent) and 30 days in rainy season (66.8 per cent) from 50 DAA to 70 DAA and 80 DAA, respectively. During this period, seed moisture content within the fruit was maintained at values close to 50 per cent, which was much higher than values recorded in matured cereal seeds reported in many development and maturation studies.

Maximum shoot length occurred 5 and 15 days after mass filling stage in summer and rainy seasons, respectively which coincided with 60 DAA in summer

season and 70 DAA in rainy season. In the present investigation, a positive correlation was obtained between seed maturity, and germination, shoot and root length, seedling dry weight and vigour index. Fruits harvested beyond 60 and 70 DAA in summer and rainy seasons, respectively yielded quality seeds that showed good strength of physiological parameters listed above.

To conclude this study, seed quality in ash gourd remained high after mass maturity (55-60 DAA) and attained maximum quality which continued upto 70 DAA in summer season and 80 DAA in rainy season. In practice, seed producers do not leave fruits on the plant for an expanded period (70-80 DAA), because of the likelihood of viviparous germination and disease or damage by pest. From the results of physiological evaluation, it would appear sensible to suggest that fruits can be harvested 60 DAA in summer season, when pedicel became dried or angular; and 70 DAA in rainy season, when fruits became thick with powdery ash and peduncular end of the fruit shoulder depressed fully. Leaving fruits beyond 80 DAA in rainy season invite fungal growth. During the final maturity stages, seed coat became dark brown, detached from the placenta, easily comes out from the pulp, when cut open and scooped. At 70 DAA, almost all the fruit pedicel portion of summer crop became dried.

Complete crop senescence occurred 170 days after sowing in summer, whereas in rainy season vine and fruit attachment with plants existed beyond 180 days.

Overall results obtained from the biochemical changes during seed development maturation proved that seed maturity starts from 60 DAA in summer season and 70 DAA in rainy season.

Seed coat development in cucurbit is peculiar during development and maturation of seed. It was evident from the microtome sections that structural changes in seed coat of matured seeds were entirely different from the seed coat of immature seeds. Elongated tubular or rod shaped thickening in epidermal cells running in the radial direction from the inside to outside, was visible in the seed coat of matured seeds of ash gourd, which was absent in the seed coat of immature seeds. In immature

seed, seed coat layers were so compact that it was difficult to split by growing embryo.

In the present study, the cotyledons developed the ability to expand first (20 DAA), followed by embryonic axis development (30-60 DAA). During embryonic axis development, hypocotyls expanded first, followed by root and shoot meristems.

The image analysis method was used to measure ash gourd seed size, shape based on perimeter, area, length, width, elongation, circularity and shape factor. All the parameters were found useful in fixing the pattern of the maturity status of seed. The data obtained were also used to find out the difference in the distinguishable seed morphological characteristics of CO 2 from Kerala local.

In rainy season, the study of seed characteristics of both the varieties using image analysis system showed that area, perimeter, length and width of the cotyledon as well as seed continued to grow and reached the highest values at 70-80 DAA, which was coincided with the stages after mass filling maturity stage. Most significant point observed here, was varietal variation, which existed among two varieties for all the stages studied starting from 20 DAA to 80 DAA.

The conclusion from this study is that using machine system good varietal discrimination could be established and growth of objects could be monitored for maturity status of the seed during development and maturation. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local confirmed their maturity stages, as both cotyledon and seeds of matured stages observed to have more values of seed descriptors.

Gel electrophoresis of protein at different stages of ash gourd seed development and maturation revealed the occurrence of matured and processed storage proteins in the bands representing seed stages of 40 to 70 and 80 DAA in summer and rainy season crops, respectively.

High molecular weight proteins were totally absent in both varieties of ash gourd, which could be observed in all the lanes of both seasons. In the present study,

identical banding pattern was reflected in both summer and rainy seasons, but varietal variation observed in Kerala local.

From the electrophoresis study, it is concluded that precise maturity detection is not possible as matured processed proteins exhibit same bands all along starting from 40 to 80 DAA in rainy season, and 40 to 70 DAA in summer season. There was no remarkable difference with respect to banding pattern among matured seeds (60-80 DAA) and moderately matured seeds (40-50 DAA). However, by and large immature stages (20-30 DAA) could be very well differentiated from that of moderately matured to matured stages.

### 6.3. INFLUENCE OF FRUIT AND SEED POSITION ON SEED QUALITY

Seeds from penduncular segment in all the fruit position showed higher moisture content in all the stages. In ash gourd, dry weight of seed increased steadily and reached a maximum at 70 DAA except for peduncular segments, in which the increase in seed dry weight was not encouraging, which was low in all the stages. The same trend continued upto 70 DAA with little variation than earlier stages. Seeds from peduncular segments of bottom, middle and top fruits gave the lowest germination. In ash gourd, seeds closer to the stem exhibited lower performance. Seeds from top fruits outperformed middle and bottom fruits. The mature seeds from top fruits were heavier than those from middle and bottom fruits. In the apical portion of the plants, fruit size may increase or decrease, but seed content will be better, consequently more seed recovery also.

From this study, it is concluded that the ash gourd fruits from the top nodes (>46<sup>th</sup> node) could be utilised for seed purpose and the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised for vegetable purpose.

### 6.4. EFFECT OF FRUIT STORAGE ON SEED QUALITY

Influence of extended storage of matured (70 DAA) ash gourd fruits under room storage condition was studied using CO 2 and Kerala local varieties. During the course of storage, there was continuous weight loss in both the varieties upto 12 months period due to water evaporation, which resulted in dehydration of fruits leaving pithy pulp and seed mass intact. Germination of seeds significantly increased

from 36.5 and 38.0 per cent at the time of storage to 94.5 and 91.5 per cent after eight months of storage in respective varieties.

The results with respect to speed of germination, shoot length, root length and vigour index were similar to that of germination during the storage. After eight to nine months of storage, there was a slight decrease in germination and vigour, indicating the on set of seed deterioration inside the fruit during storage. During unavoidable circumstances, fruit storage is recommended for post harvest maturation to achieve higher germination. Keeping fruits under storage beyond twelve months may not give benevolent effect on seed quality. If deterioration started inside the fruit, the entire lot will be wasted quickly.

# 6.5. STANDARDISATION OF A SUITABLE METHOD OF SEED EXTRACTION, SEPARATION, WASHING AND DRYING

Fermentation method is still a suitable method for seed extraction in cucurbits. But in ash gourd, proper attention on seed extraction has not been followed at all by the seed producers and growers. Fermentation of seeds along with pulp filled in 300 gauge closed polythene bags and kept for fermentation in dark room (approximately 25°C temperature for 72 h) recorded higher germination followed by open fermentation for 72h (25°C temperature) and non-fermentation methods. The beneficial effect of closed fermentation was pronounced clearly when fermentation was done followed by combination of washing for 15 min., drying in room temperature (25°C) for 72 h and sun drying (27-30°C) for 12 h. These treatments had independent beneficial effect even without storage. Significant variations were observed between closed and open fermentation vs non-fermentation method with regard to speed of germination during the initial period of storage.

The results clearly established that non-removal of mucilaginous substances in non-fermented seed which prevented germination and rate of germination. Closed fermentation for 72 h combined with washing for 15 min, drying at room temperature for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth. Even after five months of storage, closed fermentation outperformed non- fermentation method by recording 10.4 and 23.6 per cent increase in germination and speed of germination, respectively.

From this study, it is concluded that the closed fermentation for 72 h followed by water washing for 15 min, drying at room temperature for 72 h and sun drying for 12 h found to be a suitable method of seed extraction in ash gourd. This method is not only increased the seed recovery, but also maximized the seed germination and vigour. It is a easy, and no cost and low cost technology for seed extraction and separation in ash gourd.

## 6.6. ON FARM TRIAL FOR TEST VERIFICATION OF THE BEST METHOD OF SEED EXTRACTION, SEPARATION, WASHING AND DRYING

An on farm trial was conducted to test verify the performance of the best method of seed extraction in the seed producers field (Mr. K.R. Rangaraj, Kondamuthunur Village of Erode district) using 4.5 tonnes of CO 2 ash gourd fruits harvested from 2 acres. The seed recovery was 0.68 per cent, which yielded 305 kgs of good quality seed from 2 acres of CO 2 ash gourd seed crop. The net income was Rs. 57, 462.50/- per acre with the cost benefit ratio of 1:3.1.

Therefore, it is concluded that closed fermentation for 72 h combined with washing in running water for 15 min. drying under room temperature (25°C) for 72 h and sun drying for 12 h could be recommended for ash gourd seed extraction and it could be successfully adopted for large-scale seed production to ensure good quality seeds.

Seasons, seed position, fruit storage and extraction methods on seed maturity and quality maturity assessment by physico-biochemical methods in ash gourd (*BENINCASA HISPIDA COGN*.) cvs. CO 2 and Kerala local

Seasonal, seed positional influence on seed maturity, their assessment through image analysis histological, electrophoretic methods, fruit storage and closed fermentation techniques in ash gourd cvs. CO 2 and Kerala local

Seed development and maturation, influenced by season seed position fruit storage and closed fermentation of seed extraction method in ash gourd on seed quality (*Benicasu hispida* Cogn.) cvs. CO 2 and Kerala local

Table 1. Influence of season on flowering behaviour in ash gourd varieties, CO 2 and Kerala local

		CO 2			Kerala local		Season	n mean	C	D (P=0.05)	)
Flowering characteristics	Summer season	Rainy season	Mean	Summer season	Rainy season	Mean	Summer season	Rainy season	S	V	SxV
Days to male bud initiation to anthesis	5.7	2.9	4.3	5.7	2.8	4.3	5.7	2.8	0.39	NS	NS
Days to female bud initiation to anthesis	5.3	2.8	4.0	4.4	3.1	3.8	4.9	3.0	0.39	NS	0.56
Days to first male flower initiation	64.9	51.9	58.4	62.9	45.7	54.3	63.9	48.8	0.60	0.60	0.85
Days to first female flower initiation	66.3	54.0	60.2	64.7	53.5	59.1	65.5	53.8	0.72	0.72	NS
Node at which first male flower appeared	17.4	12.3	14.9	16.4	14.8	15.6	16.9	13.6	0.52	0.52	0.73
Node at which first female flower appeared	25.4	15.8	20.6	26.3	18.9	22.6	25.9	17.4	1.66	1.66	NS
Sex ratio (male to female)	20:1	17:1	19:1	22:1	17:1	20:1	21:1	17:1	-	-	-
Number of fruits per vine	1.4	4.4	2.9	1.7	5.8	3.8	1.6	5.1	0.46	0.46	0.65

Table 2. Fruit weight (g) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

Waring (N) / constant (C)							Da	ıys after a	nthesis (I	<b>)</b> )							Mean
Variety (V) / season (S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	
CO 2																	
Summer season	178	1453	1850	2862	3212	4152	4904	5235	5911	6313	6610	6812	6347	6137	-	-	3874
Rainy season	195	1135	2815	3922	4262	5005	6204	6827	7308	7514	7914	8529	8627	9104	9005	9006	6086
Mean	186	1294	2332	3392	3737	4578	5554	6031	6609	6913	7262	7670	7487	7621	4503	4502	4980
Kerala local																	
Summer season	157	1077	1521	2507	2906	3807	4303	4803	5008	5202	5308	5205	5002	4849	-	-	3229
Rainy season	183	1377	2556	2867	3896	4247	4849	5225	5576	5614	5801	5903	6219	6402	6237	6205	4579
Mean	170	1227	2088	2687	3401	4027	4576	5014	5292	5408	5554	5555	5610	5625	3119	3103	3903
Summer season mean	168	1265	1685	2685	3059	3979	4604	5019	5459	5758	5959	6009	5675	5493	-	-	3551
Rainy season mean	189	1256	2736	3395	4079	4626	5526	6026	6442	6564	6858	7216	7423	7753	7621	7605	5332
Grand mean	178	1260	2210	3040	3569	4303	5065	5522	5950	6161	6408	6612	6549	6623	3810	3802	
		I	)	V	V	,	S	D	κV	D	x S	V	x S	D x '	V x S		
SEd		12	.95	4	58		58	18	.32	18.	.32	6.			.91		
CD (P=0.05)		25	.82	9.	15	9.	15	36	.60	36.	.60	12	.94	51	.76		

Table 3. Fruit circumference (cm) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

Waite (W) / constant							Ι	Days after	anthesis	(D)							Mean
Variety (V) / season (S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	
CO 2																	
Summer season	16.1	18.5	29.5	34.4	41.0	47.2	50.3	52.1	59.0	62.2	67.5	72.2	75.1	75.0	-	-	44.6
Rainy season	24.4	25.8	30.6	35.7	43.0	48.6	52.6	54.6	69.2	76.2	76.3	76.3	86.9	87.8	85.0	84.0	59.9
Mean	20.2	22.1	30.0	35.0	42.0	47.9	51.5	53.4	64.1	70.2	71.9	74.2	81.0	86.9	42.8	42.5	52.2
Kerala local																	
Summer season	13.9	19.1	29.1	33.3	38.7	45.4	47.3	48.5	50.1	53.6	55.2	57.0	57.1	59.9	-	-	38.0
Rainy season	23.4	28.4	32.9	35.1	42.1	46.9	51.2	54.3	58.7	61.1	60.7	62.1	60.7	61.1	60.0	61.0	50.0
Mean	18.6	23.7	30.9	34.2	40.4	46.1	49.2	51.4	54.4	57.4	57.9	59.6	58.9	60.5	30.3	30.5	44.0
Summer season mean	15.0	18.8	29.3	33.9	39.8	46.3	48.8	50.3	54.6	58.9	61.4	64.6	66.1	72.9	-	-	41.3
Rainy season mean	23.9	27.0	31.7	35.4	42.6	47.7	51.9	54.4	64.0	68.0	68.4	69.2	73.8	74.4	73.1	73.0	55.0
Grand mean	19.4	23.0	30.4	34.7	41.2	47.0	50.4	52.4	59.3	63.8	65.0	66.9	69.8	73.7	36.6	36.5	
		]	D	•	V		S	Dy	ι V	D	x S	V	x S	D x V	VxS		
SEd		0.	.28	0.	10	0.	09	0.4	40	0	39	0.	14	0.3	56		
CD (P=0.05)		0.	.56	0.	19	0.	19	0.3	80	0.3	80	0.	28	1.3	13		

Table 4. Fruit length (cm) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

Variety (V) /season		asii goui	- C VIII C		z una me	<u> </u>		<u> </u>	anthesis (			, minden	eed by b	Cusons			
(S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2																	
Summer season	12.3	22.1	25.9	25.1	25.8	29.7	32.6	33.9	36.9	36.6	37.0	36.8	35.1	34.1	-	-	26.5
Rainy season	14.1	16.3	22.1	25.2	27.6	28.6	39.0	42.2	46.3	48.2	48.3	49.3	49.3	50.2	54.3	54.2	38.5
Mean	13.2	19.2	24.0	25.1	26.7	29.1	35.8	38.0	41.6	42.6	42.6	43.0	42.2	42.3	27.1	27.2	32.5
Kerala local																	
Summer season	13.5	20.0	21.1	24.6	26.1	29.4	29.9	29.9	30.1	30.2	30.1	30.8	30.9	30.1	-	-	23.5
Rainy season	11.37	18.2	24.2	27.13	28.0	28.1	38.1	39.1	39.6	39.6	38.7	39.7	40.8	40.7	40.1	41.6	33.4
Mean	12.4	19.1	22.7	25.8	27.0	28.7	34.0	34.5	34.8	34.9	34.4	35.2	35.9	35.4	20.0	20.8	28.5
Summer season mean	12.9	21.1	23.5	24.8	25.9	29.6	31.2	31.9	33.5	33.6	33.5	33.8	33.0	32.1	-	-	25.0
Rainy season mean	12.7	17.2	23.2	26.1	27.8	28.3	38.6	40.7	42.9	43.9	43.5	44.5	45.0	45.5	47.2	47.9	35.9
Grand mean	12.8	19.1	23.4	25.5	26.9	28.9	34.9	36.3	38.2	38.7	38.5	39.1	39.0	38.8	23.6	23.9	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.03	0.01	0.01	0.06	0.05	0.01	0.08
CD (P=0.05)	0.07	0.03	0.03	0.11	0.11	0.04	0.16

Table 5. Fruit diameter (cm) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

Variety (V) / seesen (C)							Ι	Days after	anthesis	(D)							Mean
Variety (V) / season (S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2																	
Summer season	6.3	6.6	15.2	16.9	18.3	18.3	19.3	20.1	21.3	22.7	23.0	23.1	23.0	23.0	-	-	16.0
Rainy season	6.4	8.7	16.5	18.1	19.5	20.1	22.1	22.1	23.3	24.4	26.5	26.6	26.0	26.1	26.1	26.2	21.1
Mean	6.3	7.7	15.9	17.5	18.9	19.2	20.7	21.1	22.3	23.5	24.7	24.8	24.5	24.6	13.0	13.0	18.6
Kerala local																	
Summer season	5.4	9.4	14.6	15.5	16.1	16.9	17.2	18.1	18.2	18.3	18.6	18.4	18.3	18.4	-	-	13.9
Rainy season	5.8	9.6	15.4	16.4	17.0	17.4	17.9	18.6	19.1	19.8	19.9	20.1	20.2	20.1	20.1	19.7	17.3
Mean	5.6	9.5	14.9	15.9	16.6	17.2	17.5	18.3	18.7	19.0	19.3	19.3	19.2	19.2	10.0	9.85	15.6
Summer season mean	5.9	7.9	14.9	16.2	17.2	17.6	18.2	19.1	19.7	20.4	20.8	20.7	20.7	20.7	-	-	15.0
Rainy season mean	6.1	9.19	15.9	17.2	18.2	18.9	20.0	20.3	21.2	22.0	23.2	23.3	23.1	23.1	23.1	22.9	19.2
Grand mean	5.9	8.6	15.4	16.7	17.8	18.2	19.1	19.8	20.5	21.3	22.0	22.0	21.9	21.9	11.6	11.5	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.08	0.03	0.03	0.12	0.12	0.04	0.17
CD (P=0.05)	0.17	0.06	0.06	0.25	0.25	0.08	0.35

Table 6. Fruit pulp thickness of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

Variaty (V) / sassan (S)							I	Days after	anthesis	(D)	•						Mean
Variety (V) / season (S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2																	
Summer season	3.4	3.9	8.5	10.7	11.5	10.9	11.5	11.4	12.5	14.1	14.3	14.7	14.0	14.1	-	-	9.7
Rainy season	4.0	4.6	12.2	12.1	13.1	13.2	14.0	14.2	14.7	15.9	17.7	18.3	17.6	17.5	17.5	17.4	11.8
Mean	3.7	4.3	10.3	11.4	12.3	12.0	12.7	12.8	13.6	15.0	15.9	16.5	15.8	15.7	-	-	10.8
Kerala local																	
Summer season	3.1	4.5	8.1	7.7	9.8	7.1	7.0	7.0	7.3	6.7	6.9	6.7	6.7	6.7	-	-	7.6
Rainy season	3.8	4.9	8.3	7.7	7.1	7.7	7.8	7.3	7.4	8.1	8.3	8.4	8.4	8.6	8.6	8.1	7.4
Mean	3.4	4.7	8.2	7.7	8.4	7.4	7.5	7.1	7.3	7.4	6.0	7.6	7.7	7.6	4.3	4.0	7.5
Summer season mean	3.3	4.3	8.3	9.2	10.7	9.0	9.2	9.3	9.8	10.4	10.6	10.8	10.8	10.4	-	-	8.7
Rainy season mean	3.8	4.8	10.2	9.9	10.1	10.4	10.8	10.7	11.0	12.0	11.4	13.4	13.0	13.1	4.3	4.0	9.6
Grand mean	3.6	4.5	9.3	9.6	10.4	9.8	10.0	10.1	10.5	11.2	11.0	12.0	13.4	11.6	2.6	2.0	

	D	V	S	D x V	D x S	VxS	D x V x S
SEd	2.39	0.84	0.85	3.38	3.40	1.19	4.78
CD (P=0.05)	4.77	1.68	NS	6.75	NS	NS	NS

Table 7. Seed cavity diameter (cm) of ash gourd varieties CO 2 and Kerala Local during seed growth and development as influenced by season

Variaty (V) / sassan (C)							I	Days after	anthesis	(D)							Mean
Variety (V) / season (S)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2																	
Summer season	1.0	3.5	4.1	6.3	6.9	7.7	8.7	8.8	9.0	8.9	8.6	8.3	7.9	8.8	-	-	6.1
Rainy season	1.4	2.9	4.3	6.1	6.4	6.8	8.1	8.2	8.6	8.5	8.9	9.0	8.4	8.4	8.8	8.5	7.0
Mean	1.2	3.2	4.1	6.2	6.7	7.3	8.3	8.5	8.7	8.8	8.9	8.6	8.1	8.5	4.4	4.3	6.6
Kerala local																	
Summer season	1.3	4.4	6.5	8.1	9.8	9.8	10.5	11.1	11.1	11.5	11.6	11.5	11.8	11.8	-	-	8.1
Rainy season	1.5	4.8	6.7	8.8	9.5	9.7	10.1	11.4	11.5	11.6	11.8	11.7	11.9	11.7	11.7	11.6	9.7
Mean	1.4	4.6	6.6	8.4	9.6	9.7	10.3	11.2	11.2	11.5	11.7	11.9	11.6	11.7	5.9	5.8	8.9
Summer season mean	1.1	3.9	5.3	7.2	8.3	8.7	9.6	9.9	9.9	10.0	10.3	9.9	9.9	10.2	-	-	7.1
Rainy season mean	1.5	3.9	5.5	7.5	8.0	8.3	9.1	9.7	9.9	10.0	10.3	10.4	10.1	10.0	10.3	10.0	8.4
Grand mean	1.3	3.9	5.4	7.4	8.1	8.5	9.4	9.8	9.9	10.1	10.2	10.1	10.0	10.1	5.1	5.0	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.05	0.02	0.02	0.08	0.08	0.05	0.10
CD (P=0.05)	0.10	0.04	0.04	0.15	0.15	0.10	0.21

Table 8. Number of well filled good seeds in Ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) / season (S)	Days after anthesis (D)													Mean
variety (v)/ season (s)	20	25	30	35	40	45	50	55	60	65	70	75	80	Ivican
CO 2														
Summer season	125	310	618	814	818	703	835	636	835	993	920	-	-	586
Rainy season	151	373	402	506	603	447	894	833	863	900	991	831	833	664
Mean	138	342	510	660	711	575	865	735	850	947	956	416	417	624
Kerala local														
Summer season	300	572	927	1277	1400	2450	2441	2353	2515	2001	2805	-	-	1465
Rainy season	431	587	833	1539	2235	2329	2451	2555	2204	2424	2503	2604	2609	1946
Mean	366	580	880	1408	1817	2390	2446	2454	2360	2212	2654	1302	1305	1705
Summer season mean	213	441	773	1046	1109	1577	1638	1495	1675	1497	1863	-	-	1025
Rainy season mean	291	480	618	1022	1419	1388	1672	1694	1534	1662	1747	1717	1721	1305
Grand mean	252	461	695	1034	1264	1482	1655	1594	1604	1579	1605	858	860	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	11.0	4.3	4.4	15.7	15.6	6.15	22.19
CD (P=0.05)	22.2	8.7	8.7	31.4	31.5	12.3	44.5

Table 9. Fresh weight (g) of seeds of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) / season (S)	Days after anthesis (D)												Mean	
variety (v)/ season (s)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	35.2	40.0	64.1	62.3	62.2	64.9	70.0	84.0	82.3	80.2	80.1	-	-	56.0
Rainy season	42.2	45.1	68.5	76.3	77.6	79.3	82.5	88.1	92.6	91.5	92.3	92.2	91.6	78.5
Mean	38.7	42.6	66.3	69.3	69.9	72.1	76.2	86.1	87.5	87.4	86.2	46.6	45.8	67.3
Kerala local														
Summer season	56.1	89.3	89.4	98.1	102.1	99.9	102.9	103.3	103.9	97.8	93.1	-	-	79.7
Rainy season	83.1	102.8	105.1	135.1	155.1	169.15	183.7	188.4	181.2	183.2	182.2	180.1	176.6	155.8
Mean	69.6	96.0	97.2	116.6	128.6	134.5	143.0	145.8	142.5	140.5	137.7	90.0	88.3	117.8
Summer season mean	45.7	64.7	76.7	80.2	80.2	82.4	86.5	98.7	93.1	90.5	86.67	-	-	67.9
Rainy season mean	62.7	74.0	86.8	105.7	116.4	124.2	133.1	138.2	136.9	137.3	137.2	136.6	134.0	117.2
Grand mean	54.1	69.3	81.8	92.9	99.3	103.3	109.8	115.9	115.9	115.0	139.9	111.9	68.3	67.0

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.02	0.01	0.01	0.04	0.04	0.01	0.05
CD (P=0.05)	0.05	0.02	0.02	0.07	0.08	0.03	0.11

Table 10. Dry weight (g) of seeds of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Veriety (V) / seesen (S)	Days after anthesis (D)											Moon		
Variety (V) / season (S)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	14.2	16.3	31.0	31.0	31.0	32.0	34.0	36.4	34.9	35.6	33.1	-	-	27.3
Rainy season	28.2	28.0	33.1	34.6	34.9	33.5	36.2	39.2	41.0	40.0	39.5	37.7	39.1	33.9
Mean	21.2	22.2	32.0	32.8	33.0	32.8	35.1	37.8	38.0	37.9	36.3	18.9	19.6	30.6
Kerala local														
Summer season	20.0	26.0	37.1	49.1	52.0	48.9	49.6	72.0	72.1	69.0	55.0	-	-	47.6
Rainy season	42.2	42.4	55.4	60.1	66.1	86.3	94.9	108.1	103.0	105.0	103.0	100.6	100	76.9
Mean	31.1	34.2	46.2	54.9	59.1	67.6	72.2	90.0	87.6	87.0	79.0	50.3	50.0	62.3
Summer season mean	17.1	21.1	35.1	42.1	41.5	40.5	41.8	54.2	53.6	52.3	44.1	-	-	37.4
Rainy season mean	35.2	35.1	43.2	45.6	50.6	59.9	65.6	73.7	72.0	72.6	71.3	69.1	69.6	55.4
Grand mean	26.2	28.2	39.2	43.9	46.0	50.2	53.7	63.9	62.8	62.5	57.7	34.6	34.8	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.02	0.007	0.006	0.03	0.03	0.009	0.04
CD (P=0.05)	0.04	0.02	0.02	0.05	0.05	0.02	0.07

Table 11. Seed Moisture content (%) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variata (VI) / account (C)	Days after anthesis (D)										Massa			
Variety (V) / season (S)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	60.6	59.5	51.5	49.9	50.1	49.9	51.4	56.6	57.3	57.0	58.2	-	-	43.2
Rainy season	66.1	63.7	51.3	54.4	54.6	57.6	55.8	55.4	55.4	56.6	67.0	59.0	57.3	57.2
Mean	63.3	61.6	51.4	52.1	52.3	53.7	53.6	56.0	56.3	56.5	57.6	59.0	57.3	50.2
Kerala local														
Summer season	56.7	52.6	38.5	38.7	49.3	51.0	51.8	30.2	30.8	30.1	40.9	-	-	33.8
Rainy season	75.7	74.8	64.9	63.6	57.3	48.6	48.8	42.6	43.1	42.5	43.4	42.8	43.3	53.2
Mean	66.2	63.7	51.7	51.2	53.3	49.8	50.3	36.5	36.9	36.3	42.1	42.8	43.3	43.5
Summer season mean	58.7	56.0	45.0	44.3	49.7	50.4	51.6	43.4	44.0	43.6	43.6	-	-	38.5
Rainy season mean	70.9	69.3	58.1	59.0	50.9	53.0	52.3	49.0	49.2	49.2	50.2	50.9	50.3	55.2
Grand mean	64.8	62.7	51.6	51.7	52.8	51.8	52.0	46.2	46.7	46.4	49.9	50.9	50.3	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.22	0.09	0.09	0.40	0.40	0.30	0.44
CD (P=0.05)	0.50	0.20	0.20	0.80	0.70	0.60	0.90

Table 12. Hundred seed weight (g) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) / season (S)  Days after anthesis (D)														Mann
variety (v) / season (s)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	2.16	2.18	2.23	3.21	3.95	4.33	4.91	5.42	5.49	5.89	5.86	-	-	3.51
Rainy season	2.26	2.31	3.30	4.40	4.71	5.61	5.73	5.96	5.90	6.11	6.14	7.70	6.72	5.14
Mean	2.21	2.24	2.77	3.80	4.33	4.97	5.32	5.69	5.70	6.00	6.00	3.85	3.36	4.33
Kerala local														
Summer season	3.33	3.74	4.15	4.29	5.37	5.61	5.76	5.83	5.78	5.91	6.83	-	-	4.35
Rainy season	3.15	3.91	4.31	4.62	6.32	6.35	6.39	6.85	7.56	8.61	9.71	9.05	9.06	6.61
Mean	3.24	3.82	4.23	4.45	5.84	5.98	6.08	6.34	6.67	7.26	8.27	4.52	4.53	5.48
Summer season mean	2.74	2.96	3.19	3.75	4.66	4.97	5.33	5.62	5.63	5.90	6.34	-	-	3.93
Rainy season mean	2.70	3.11	3.81	4.51	5.52	5.98	6.06	6.40	6.73	7.36	7.93	8.37	7.89	5.87
Grand mean	2.72	3.03	3.50	4.13	5.09	5.47	5.70	6.01	6.18	6.63	7.14	4.18	3.94	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.005	0.002	0.002	0.007	0.007	0.003	0.009
CD (P=0.05)	0.01	0.004	0.004	0.014	0.014	0.006	0.02

Table 13. Seed recovery (%) of Ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) / season (S)	Days after anthesis (D)												Mean	
variety (v) / season (s)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	0.36	0.38	0.66	0.56	0.51	0.46	0.48	0.50	0.48	0.46	0.43	-	_	0.52
	(3.5)	(3.6)	(4.7)	(4.3)	(4.1)	(3.9)	(3.9)	(4.0)	(3.9)	(3.9)	(3.8)	-	-	(3.8)
Rainy season	0.51	0.51	0.74	0.63	0.59	0.54	0.54	0.55	0.51	0.56	0.55	0.43	0.41	0.48
	(4.1)	(4.1)	(4.9)	(4.6)	(4.4)	(4.2)	(4.2)	(4.3)	(4.1)	(4.3)	(4.3)	(3.8)	(3.7)	(3.9)
Mean	0.67	0.45	0.74	0.60	0.55	0.50	0.51	0.53	0.50	0.51	0.49	0.20	0.21	0.49
	(4.6)	(3.8)	(4.8)	(4.4)	(4.3)	(4.0)	(4.1)	(4.1)	(4.0)	(4.1)	(4.0)	(1.84)	(1.90)	(3.9)
Kerala local														
Summer season	0.69	0.66	0.87	1.0	1.28	0.97	0.95	1.4	1.4	1.4	1.1	-	-	1.2
	(4.8)	(4.7)	(5.4)	(5.8)	(6.5)	(5.7)	(5.6)	(6.7)	(6.8)	(6.7)	(6.1)			(5.6)
Rainy season	1.7	1.45	1.40	1.39	1.81	1.56	1.68	1.85	1.73	1.68	1.59	1.60	1.61	1.37
	(7.4)	(6.9)	(6.8)	(6.8)	(7.7)	(7.1)	(7.5)	(7.8)	(7.6)	(7.5)	(7.2)	(7.3)	(7.3)	(6.6)
Mean	1.17	1.06	1.14	1.20	1.54	1.27	1.32	1.60	1.50	1.53	1.36	0.80	0.80	1.25
	(6.0)	(5.8)	(6.0)	(6.3)	(7.1)	(6.4)	(6.5)	(7.2)	(7.1)	(7.0)	(6.7)	(3.6)	(3.6)	(6.1)
Summer season mean	0.53	0.52	0.77	0.78	0.78	0.76	0.75	0.95	0.95	0.96	0.84	-	-	0.83
	(4.1)	(4.1)	(5.0)	(5.0)	(5.3)	(4.9)	(4.9)	(5.5)	(5.4)	(5.5)	(5.1)			(4.7)
Rainy season mean	1.31	0.98	1.07	1.01	1.20	1.01	1.08	1.18	1.11	1.07	1.01	1.01	1.02	0.92
	(6.5)	(5.5)	(5.9)	(5.7)	(6.0)	(5.5)	(5.7)	(5.9)	(5.8)	(5.7)	(5.5)	(5.5)	(5.5)	(5.3)
Grand mean	0.92	0.75	0.92	0.90	1.05	1.08	0.91	1.06	1.03	1.02	0.92	0.50	0.51	
	(5.3)	(4.8)	(5.4)	(5.4)	(5.7)	(5.2)	(5.3)	(5.7)	(5.6)	(5.6)	(5.3)	(2.7)	(2.8)	

	D	V	S	D x V	D x S	V x S	DxVxS
SEd	0.02	0.006	0.006	0.02	0.02	0.009	0.03
CD (P=0.05)	0.04	0.012	0.012	0.04	0.05	0.018	0.07

(Figures in parentheses are arc-sine transformed values)

Table 14. Embryo thickness of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Veriety (V) / seesen (S)						Days af	ter anthesis	s (D)						Mean
Variety (V) / season (S)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	0.11	0.32	0.62	0.71	0.80	0.95	1.34	1.53	1.80	1.79	1.83	-	-	0.90
Rainy season	0.18	0.45	0.65	0.70	0.86	0.97	1.37	1.60	1.85	1.89	1.93	1.96	1.98	1.26
Mean	0.15	0.39	0.64	0.70	0.8.	0.96	1.35	1.56	1.83	1.84	1.88	0.98	0.99	1.08
Kerala local														
Summer season	0.16	0.26	0.66	0.70	0.84	1.01	1.39	1.40	1.95	1.95	2.01	-	-	0.95
Rainy season	0.20	0.30	0.77	0.85	0.98	1.24	1.49	1.86	1.99	2.06	2.11	2.13	2.18	1.40
Mean	0.18	0.28	0.72	0.78	0.91	1.13	1.44	1.63	1.97	2.00	2.06	1.06	1.09	1.17
Summer season mean	0.14	0.29	0.64	0.70	0.82	0.98	1.36	1.46	1.88	1.87	1.92	-	-	0.93
Rainy season mean	0.20	0.38	0.71	0.78	0.92	1.10	1.43	1.73	1.92	1.97	2.02	2.05	2.08	1.33
Grand mean	0.16	0.33	0.68	0.74	0.87	1.04	1.39	1.60	1.90	1.92	1.97	1.02	1.04	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.007	0.003	0.003	0.009	0.009	0.004	0.013
CD (P=0.05)	0.014	0.006	0.006	0.02	0.02	0.008	0.026

Table 15. Embryo weight of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) / seesan (S)						Days af	ter anthesis	s (D)						Mean
Variety (V) / season (S)	20	25	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2														
Summer season	0.010	0.011	0.031	0.042	0.063	0.073	0.11	0.12	0.14	0.14	0.15	-	-	0.07
Rainy season	0.010	0.021	0.042	0.052	0.063	0.084	0.121	0.131	0.142	0.142	0.152	0.152	0.154	0.0975
Mean	0.010	0.016	0.037	0.047	0.063	0.079	0.116	0.126	0.142	0.142	0.152	0.076	0.0763	0.08
Kerala local														
Summer season	0.021	0.031	0.420	0.063	0.073	0.084	0.131	0.142	0.152	0.163	0.173	-	-	0.083
Rainy season	0.021	0.310	0.052	0.063	0.084	0.105	0.142	0.163	0.170	0.163	0.184	0.185	0.184	0.120
Mean	0.021	0.031	0.047	0.063	0.079	0.094	0.137	0.153	0.161	0.163	0.179	0.092	0.092	0.10
Summer season mean	0.016	0.021	0.037	0.052	0.068	0.788	0.121	0.131	0.147	0.152	0.163	-	-	0.080
Rainy season mean	0.016	0.026	0.047	0.058	0.073	0.094	0.131	0.147	0.156	0.152	0.168	0.168	0.168	0.110
Grand mean	0.016	0.024	0.042	0.055	0.071	0.087	0.126	0.139	0.152	0.153	0.166	0.084	0.084	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.002	0.001	0.0007	0.003	0.003	0.001	0.004
CD (P=0.05)	0.004	0.002	0.002	0.005	0.005	0.002	0.007

Table 16. Speed of germination of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variaty (V) / sassan (S)		8			Days a	after anthesi	s (D)					Mean
Variety (V) / season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	0.53	5.8	10.1	12.1	13.0	13.1	14.4	13.9	12.9	-	-	8.7
Rainy season	0.0	3.16	5.9	10.1	11.0	12.7	13.3	13.9	14.4	14.4	13.9	10.3
Mean	0.3	4.5	8.0	11.1	12.0	12.9	13.9	13.9	13.7	7.2	6.9	9.5
Kerala local												
Summer season	0.15	0.15	5.8	9.7	10.1	12.7	13.1	13.1	12.1	-	-	6.9
Rainy season	0.0	0.15	0.52	5.8	9.3	10.1	12.1	12.3	13.1	13.1	13.3	8.2
Mean	0.08	0.15	3.1	7.7	9.7	11.4	12.6	12.7	12.6	6.5	6.7	7.6
Summer season mean	0.34	2.9	7.9	10.9	11.6	12.9	13.8	13.5	12.5	-	-	7.9
Rainy season mean	0.0	1.7	3.2	7.9	10.2	11.4	12.7	13.0	13.8	13.8	13.6	9.2
Grand mean	0.17	2.3	5.6	9.4	10.9	12.1	13.2	13.3	13.1	6.8	6.8	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.04	0.02	0.02	0.06	0.06	0.02	0.08
CD (P=0.05)	0.08	0.04	0.04	0.11	0.11	0.05	0.16

Table 17. Germination (%) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variaty (V) Jacque (C)	Days after anthesis (D)  30 35 40 45 50 55 60 65 70 75 80											
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	4.0	10.6	35.0	42.5	53.5	73.5	83.5	85.5	82.5	-	-	42.8
	(11.5)	(18.9)	(36.3)	(40.7)	(47.0)	(59.0)	(66.0)	(67.6)	(65.3)			(37.5)
Rainy season	0	17.5	31.9	31.9	55.5	76.0	74.5	85.5	86.5	84.5	85.0	57.1
	(0.0)	(24.7)	(34.4)	(34.4)	(48.1)	(60.7)	(59.7)	(67.6)	(68.4)	(66.8)	(67.2)	(48.4)
Mean	2.0	14.0	33.4	37.2	54.5	74.8	79.0	85.0	84.0	42.2	42.5	49.9
	(5.8)	(21.8)	(33.3)	(37.5)	(47.6)	(59.8)	(62.8)	(67.6)	(66.9)	(33.4)	(33.6)	(42.9)
Kerala local												
Summer season	2.5	13.5	13.0	16.5	43.0	51.0	78.0	80.5	70.5	-	-	33.5
	(9.5)	(21.5)	(21.1)	(23.9)	(40.9)	(45.6)	(62.0)	(63.8)	(57.1)			(31.3)
Rainy season	0.0	0.53	5.3	33.3	41.5	43.5	69.4	72.5	78	81.5	84.0	46.3
	(0.0)	(4.1)	(13.4)	(35.2)	(40.1)	(41.3)	(56.4)	(58.4)	(62.0)	(64.5)	(66.4)	(40.1)
Mean	1.3	7.0	9.2	24.9	42.2	47.2	73.7	76.5	74.2	40.7	42.0	39.9
	(4.5)	(12.8)	(17.2)	(29.6)	(40.5)	(43.4)	(59.2)	(61.0)	(59.6)	(32.3)	(33.2)	(35.8)
Summer season mean	3.3	12.0	24.0	29.5	48.2	62.2	80.8	83.0	76.5	-	-	38.1
	(10.3)	(20.2)	(28.7)	(32.3)	(43.9)	(52.3)	(64.0)	(65.7)	(61.2)			(34.4)
Rainy season mean	0.0	9.0	18.6	32.6	48.5	59.7	71.9	79.0	82.2	83.0	84.5	51.7
	(0.0)	(14.5)	(23.9)	(34.8)	(44.1)	(50.9)	(58.0)	(62.9)	(65.2)	(65.7)	(66.8)	(44.3)
Grand mean	1.6	10.5	21.3	31.0	48.3	61.0	76.3	81.0	79.4	41.5	42.2	
	(5.1)	(17.3)	(26.3)	(33.6)	(44.0)	(51.6)	(61.0)	(64.3)	(63.2)	(32.8)	(33.4)	
		D	V	S	D x V	D x S	V x S	Dx	V x S			
SE	d	0.53	0.22	0.23	0.75	0.75	0.32	1.	06			

1.50

1.50

0.64

2.13

(Figures in parentheses are arc-sine transformed values)
Table 18. Shoot length (cm) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by seasons

0.45

CD (P=0.05)

1.06

0.45

Variaty (V) /seesen (S)					Days a	after anthesi	s (D)					Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	4.1	6.4	12.7	13.2	15.6	16.3	16.4	16.3	16.1	-	-	10.7
Rainy season	0.0	6.9	13.9	15.9	17.6	15.1	16.5	17.6	18.3	18.5	17.6	14.4
Mean	2.07	6.7	13.4	14.6	16.6	15.8	16.4	16.9	17.2	9.2	8.8	12.5
Kerala local												
Summer season	1.8	4.2	13.3	13.5	13.9	15.6	17.9	16.0	16.1			10.2
Rainy season	0.0	9.7	10.9	11.0	12.0	14.1	16.8	16.4	16.6	18.0	18.4	13.0
Mean	0.9	6.9	12.1	12.3	12.9	14.8	17.3	16.2	16.4	9.0	9.1	11.6
Summer season mean	2.9	5.3	13.0	13.5	14.8	15.9	17.1	16.2	16.1	-	-	10.4
Rainy season mean	0.0	8.3	12.4	13.5	14.8	14.6	16.6	17.0	17.5	18.3	17.9	13.7
Grand mean	1.5	6.8	12.7	13.4	14.8	15.3	16.9	16.6	16.8	9.1	8.9	

	D	V	S	D x V	D x S	V x S	DxVxS	
SEd	0.04	0.02	0.02	0.06	0.06	0.03	0.08	
CD (P=0.05)	0.08	0.04	0.04	0.11	0.11	0.06	0.16	

Table 19. Root length (cm) of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Moriety (W) /seesen (S)					Days	after anthesi	s (D)					- Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	12.6	13.1	14.9	15.7	16.1	16.3	18.7	18.2	18.1	-	-	13.0
Rainy season	0.0	9.7	16.1	16.9	18.3	18.3	18.9	18.8	18.8	18.7	18.9	15.8
Mean	6.3	11.4	15.5	16.4	17.2	17.3	18.2	18.5	18.4	9.4	9.5	14.4
Kerala local												
Summer season	4.3	8.8	10.5	15.1	15.1	18.0	18.1	18.0	16.9	-	-	11.4
Rainy season	0.0	6.2	12.0	16.3	15.7	17.1	18.0	18.1	18.2	18.1	18.2	14.4
Mean	2.1	7.5	11.3	15.7	15.3	17.6	18.0	18.1	17.5	9.0	9.0	12.8
Summer season mean	8.4	10.9	12.7	15.4	15.6	17.1	18.4	18.1	17.5	-	-	12.2
Rainy season mean	0.0	7.9	14.0	16.6	16.9	17.7	18.5	18.4	18.5	18.5	18.4	15.0
Grand mean	4.2	9.4	13.4	16.0	16.3	17.4	18.4	18.3	17.9	9.2	9.3	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.07	0.03	0.03	0.10	0.10	0.04	0.14
CD (P=0.05)	0.15	0.06	0.06	0.20	0.20	0.09	0.30

Table 20. Dry weight of seedlings (mg/10<sup>-1</sup>) of Ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) /seesen (S)					Days a	after anthesi	s (D)					Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Wiean
CO 2												
Summer season	13.8	14.7	25.1	31.4	32.5	33.5	36.9	34.5	34.2	-	-	23.3
Rainy season	0.0	16.9	33.1	36.1	38.7	38.1	42.8	40.1	41.9	42.2	42.5	33.9
Mean	6.9	15.8	29.1	33.8	35.6	35.8	39.9	37.3	38.0	22.1	21.2	28.6
Kerala local												
Summer season	5.43	15.0	27.5	29.8	30.2	33.0	38.2	36.1	31.0	-	-	22.4
Rainy season	0.0	16.1	29.9	31.7	32.7	33.7	38.2	40.1	41.3	40.3	41.3	31.4
Mean	2.7	15.6	28.7	30.7	31.4	33.4	38.2	38.1	36.2	20.1	20.6	26.9
Summer season mean	9.6	14.9	26.2	30.6	31.4	33.3	37.6	35.3	32.6	-	-	22.9
Rainy season mean	0.0	16.5	31.5	33.9	35.7	35.9	40.5	40.1	41.6	41.3	41.9	32.6
Grand mean	4.8	15.7	22.9	32.3	33.5	34.6	39.0	37.7	37.1	20.6	20.9	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.10	0.04	0.04	0.15	0.15	0.06	0.20
CD (P=0.05)	0.20	0.09	0.09	0.30	0.30	0.13	0.42

Table 21. Vigour index of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) /season (S)	Days after anthesis (D)											
	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	62.0	207	967	1230	1573	2398	2931	2958	2827	-	-	1378
Rainy season	0	291	959	1048	1997	2582	2644	3110	3215	2998	3092	1995
Mean	31	249	963	1139	1785	2490	2788	3034	3021	1499	1546	1686
Kerala local												
Summer season	15	176	309	474	1249	1709	2813	2762	2335	-	-	1077
Rainy season	0	854	123	910	1150	1359	2416	2503	2707	2951	3070	1640
Mean	76	514	215	692	1199	1534	2614	2632	2520	1476	1535	1358
Summer season mean	39	191	638	851	1410	2053	2872	2860	2581	-	-	1227
Rainy season mean	0	572	540	979	1574	1971	2530	2806	2961	2974	3081	1817
Grand mean	19	382	589	916	1492	2012	2701	2833	2771	1487	1540	
		D	V	S	D x V	D x S	VxS	D x '	V x S			

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	30.4	12.9	12.9	42.9	42.9	18.3	60.7
CD (P=0.05)	61.1	26.0	26.0	86.5	86.5	36.9	122.3

Table 22. Tetrazolium viability (%) test of ash gourd varieties CO 2 and Kerala local during seed growth and development as influenced by season

Variety (V) /season (S)					Days	after anthesis	s (D)					Mean
variety (v)/season (s)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	12.5	14.0	43.0	52.5	62.5	86.5	88.0	92.5	66.0	-	-	47.0
	(20.7)	(21.9)	(40.9)	(46.4)	(52.2)	(68.4)	(69.7)	(74.1)	(54.3)			(40.8)
Rainy season	0.0	15.5	40.0	56.5	66.5	85.5	85.5	86.5	100	100	100	66.9
	(0)	(23.1)	(39.2)	(48.7)	(54.6)	(67.6)	(67.6)	(68.4)	(90.0)	(90.0)	(90.0)	(58.1)
Mean	6.3	14.8	41.5	54.5	64.5	86.0	86.8	89.5	83.0	50.0	50.0	56.9
	(10.3)	(22.5)	(40.1)	(47.6)	(53.4)	(68.0)	(68.7)	(71.2)	(72.2)	(45.0)	(45.0)	(49.5)
Kerala local												
Summer season	7.5	9.0	36.5	45.5	53.5	66.5	82.5	84.5	66.5	-	-	41.0
	(15.8)	(17.4)	(37.2)	(42.4)	(47.0)	(54.6)	(65.3)	(66.8)	(54.6)			(36.5)
Rainy season	0.0	14.5	34.5	34.0	41.5	57.5	66.5	75.0	92.5	94.5	94.5	55.0
	(0.0)	(22.4)	(35.9)	(35.7)	(40.1)	(49.3)	(54.6)	(60.0)	(74.1)	(76.4)	(76.4)	(47.7)
Mean	3.8	11.8	35.5	39.8	47.5	62.0	74.5	79.8	79.5	47.3	47.3	48.0
	(7.9)	(19.9)	(36.5)	(39.0)	(43.6)	(51.9)	(59.9)	(63.4)	(64.4)	(38.2)	(38.2)	(42.1)
Summer season mean	10.0	11.5	39.8	49.0	58.0	76.5	85.2	88.5	66.3	-	-	44.0
	(18.2)	(19.7)	(39.0)	(44.4)	(49.6)	(61.5)	(67.5)	(70.5)	(54.5)			(38.6)
Rainy season mean	0	15.0	37.3	45.3	54.0	71.5	76.0	80.8	96.2	97.3	97.3	60.9
	(0)	(22.3)	(37.6)	(42.2)	(47.4)	(58.4)	(61.2)	(64.2)	(82.0)	(83.2)	(83.2)	(52.9)
Grand mean	5.0	13.3	38.5	47.1	56.0	74.0	80.6	84.6	81.2	48.6	48.6	
	(9.1)	(21.2)	(38.3)	(43.3)	(48.4)	(60.0)	(64.3)	(67.3)	(68.5)	(41.6)	(41.6)	
		-	**	~	D 11	<b>7</b> . 0	** ~					
		D	V	S	D x V	D x S	VxS		V x S			
	SEd	0.50	0.22	0.22	0.72	0.72	0.30		02			
	CD (P=0.05)	1.02	0.43	0.44	1.50	1.45	0.62	2.	05			

(Figures in parentheses are arc-sine transformed values)

Table 38. Shape factor of embryo with different stages of maturity as measured in Image Analysis system for ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variety (V) /season (S)	Days after anthesis (D)									
	20	30	40	50	60	70	80	Mean		
CO 2										
Summer season	1.19	1.16	1.21	1.18	1.17	1.17	-	1.01		
Rainy season	1.16	1.14	1.16	1.14	1.16	1.15	1.17	1.15		
Mean	1.18	1.15	1.19	1.17	1.17	1.16	0.58	1.09		
Kerala local										
Summer season	1.26	1.23	1.19	1.29	1.18	1.18	-	1.05		
Rainy season	1.23	1.22	1.21	1.21	1.21	1.19	1.21	1.21		
Mean	1.25	1.22	1.20	1.25	1.20	1.18	0.60	1.13		
Summer season mean	1.23	1.19	1.20	1.245	1.18	1.18	-	1.03		
Rainy season mean	1.12	1.18	1.19	1.18	1.18	1.17	1.19	1.19		
Grand mean	1.21	1.16	1.19	1.20	1.18	1.18	0.59			
							•			
	D	V	S	D x V	D x S	V x S	D x V x S			
SEd	0.02	0.01	0.01	0.03	0.03	0.01	0.03			
CD (P=0.05)	0.04	0.02	0.02	NS	0.05	NS	NS			

TABLE 39. SHAPE FACTOR OF SEEDS WITH DIFFERENT STAGES OF MATURITY AS MEASURED IN IMAGE ANALYSIS SYSTEM FOR ASH GOURD VARIETIES CO 2 AND KERALA LOCAL RAISED IN SUMMER AND RAINY SEASONS

Variety (V) /cooper (C)			Days	s after anthesis	(D)			Moon
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	1.18	1.16	1.16	1.18	1.16	1.15	-	0.99
Rainy season	1.21	1.17	1.12	1.12	1.12	1.12	1.13	1.14
Mean	1.19	1.17	1.14	1.15	1.14	1.14	0.57	1.07
Kerala local								
Summer season	1.17	1.16	1.17	1.14	1.14	1.16	-	0.99
Rainy season	1.17	1.16	1.21	1.15	1.15	1.14	1.16	1.16
Mean	1.19	1.17	1.14	1.15	1.14	1.14	0.57	1.08
Summer season mean	1.18	1.16	1.16	1.16	1.15	1.15	-	0.99
Rainy season mean	1.19	1.17	1.17	1.15	1.14	1.13	1.15	1.15
Grand mean	1.18	1.16	1.17	1.15	1.14	1.14	0.57	
	D	V	S	D x V	D x S	V x S	D x V x S	
SEd	0.02	0.01	0.01	0.01	0.03	0.02	0.03	
CD (P=0.05)	0.04	0.02	0.02	NS	0.05	NS	NS	

Table 23. Oil content of seed in ash gourd seed varieties CO 2 and Kerala Local during growth and development influenced by season

Variaty (V) /cascan (S)					Days a	after anthesi	s (D)					Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Wiean
CO 2												
Summer season	0.215	0.145	0.305	0.394	0.481	0.505	0.515	0.558	0.691	-	-	0.621
Rainy season	0.181	0.135	0.215	0.291	0.415	0.445	0.531	0.615	0.705	0.725	0.755	0.456
Mean	0.198	0.140	0.260	0.342	0.448	0.475	0.523	0.098	0.698	0.363	0.378	0.538
Kerala local												
Summer season	0.205	0.155	0.305	0.145	0.550	0.505	0.605	0.705	0.805	-	-	0.367
Rainy season	0.191	0.145	0.225	0.315	0.465	0.491	0.581	0.705	0.785	0.758	0.820	0.501
Mean	0.198	0.150	0.265	0.230	0.508	0.528	0.593	0.705	0.795	0.393	0.410	0.478
Summer season mean	0.210	0.150	0.305	0.270	0.516	0.535	0.560	0.614	0.748	-	-	0.490
Rainy season mean	0.186	0.140	0.220	0.303	0.440	0.468	0.556	0.660	0.745	0.755	0.788	0.480
Grand mean	0.198	0.145	0.263	0.286	0.478	0.501	0.558	0.401	0.746	0.378	0.394	

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.03	0.01	0.01	0.04	0.04	0.02	0.06
CD (P=0.05)	0.06	NS	NS	NS	NS	NS	NS

Table 24. Dehydrogenase activity (OD Value) of ash gourd seed varieties CO 2 and Kerala local during growth and development influenced by season

Variaty (VI) /sassan (S)					Days a	after anthesi	s (D)					Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	0.00	0.031	0.048	0.051	0.064	0.084	0.091	0.096	0.097	-	-	0.051
Rainy season	0.00	0.037	0.052	0.064	0.069	0.077	0.094	0.096	0.096	0.096	0.096	0.070
Mean	0.00	0.034	0.045	0.058	0.067	0.080	0.092	0.096	0.097	0.048	0.046	0.061
Kerala local												
Summer season	0.00	0.051	0.053	0.054	0.062	0.073	0.48	0.090	0.091	-	-	0.086
Rainy season	0.00	0.046	0.062	0.070	0.074	0.080	0.094	0.096	0.098	0.096	0.092	0.109
Mean	0.00	0.049	0.057	0.062	0.068	0.076	0.076	0.080	0.092	0.048	0.047	0.08
Summer season mean	0.00	0.041	0.050	0.053	0.063	0.079	0.073	0.091	0.092	-	-	0.069
Rainy season mean	0.00	0.41	0.057	0.067	0.071	0.079	0.090	0.091	0.095	0.096	0.092	0.090
Grand mean	0.00	0.041	0.054	0.059	0.067	0.078	0.078	0.079	0.090	0.056	0.056	0.045

	D	V	S	D x V	D x S	V x S	D x V x S
SEd	0.06	0.02	0.03	0.08	0.08	0.04	0.12
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS

Table 25. Protein content (%) of ash gourd seed varieties CO 2 and Kerala local during growth and development influenced by season

Variaty (V) /sassan (C)					Days	after anthesi	s (D)					Mean
Variety (V) /season (S)	30	35	40	45	50	55	60	65	70	75	80	Mean
CO 2												
Summer season	25.6	32.8	33.8	35.2	36.9	37.7	37.0	36.9	37.8	-	-	28.5
	(30.4)	(34.9)	(35.5)	(36.4)	(37.4)	(37.8)	(37.5)	(37.4)	(37.9)			(29.6)
Rainy season	27.2	32.4	35.2	36.8	37.2	34.9	37.2	37.0	37.0	38.2	30.1	34.8
	(31.4)	(34.6)	(36.4)	(37.3)	(37.6)	(36.2)	(37.6)	(37.4)	(37.5)	(38.1)	(33.1)	(36.1)
Mean	26.4	32.5	34.5	35.9	37.0	36.3	37.1	36.9	37.4	19.1	15.0	31.7
	(30.8)	(34.8)	(35.9)	(36.8)	(37.5)	(37.0)	(37.5	(37.4)	(37.7)	(19.0)	(16.6)	(33.1)
Kerala local												
Summer season	26.2	33.7	35.6	36.5	38.4	38.4	38.5	37.2	36.4	-	-	29.1
	(30.8)	(35.5)	(36.6)	(37.1)	(38.2)	(38.2)	(38.3)	(37.6)	(37.1)			(29.9)
Rainy season	27.6	33.9	34.7	36.2	36.9	38.2	38.0	36.9	36.9	37.7	38.4	35.9
	(31.6)	(35.6)	(36.0)	(37.0)	(37.4)	(38.1)	(38.0)	(37.4)	(37.4)	(37.8)	(38.3)	(36.8)
Mean	26.9	33.7	35.1	36.4	37.7	38.3	38.2	37.0	36.7	18.8	19.2	32.6
	(31.2)	(35.5)	(36.3)	(37.1)	(37.8)	(38.2)	(38.2)	(37.5)	(37.2)	(18.9)	(19.1)	(34.6)
Summer season mean	25.9	33.2	34.6	35.8	37.6	38.0	37.7	37.0	37.1	-	-	28.8
	(30.6)	(35.1)	(36.0)	(36.8)	(37.8)	(38.0)	(37.8)	(37.5)	(37.5)			(29.7)
Rainy season mean	27.4	33.1	34.9	36.5	37.0	36.6	37.6	36.9	36.9	38.0	34.3	35.0
	(31.5)	(35.1)	(36.2)	(37.1)	(37.5)	(37.2)	(37.8)	(37.4)	(37.5)	(38.0)	(35.7)	(36.5)
Grand mean	26.7	33.1	34.8	36.2	37.3	37.3	37.7	37.0	37.0	18.9	17.13	
	(31.0)	(35.1)	(36.1)	(36.9)	(37.6)	(37.6)	(37.8)	(37.5)	(37.5)	(19.0)	(17.9)	
		D	V	S	D x V	D x S	V x S	D x '	V x S			
\$	SEd	1.0	0.46	0.46	1.54	1.54	0.66	2.	19			
(	CD (P=0.05)	2.2	NS	0.94	NS	3.12	NS	N	IS			

Table 26. Area (cm²) of embryo as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala Local raised during seed growth and development in summer and rainy seasons

Mariata (M) (access (C)			Day	ys after anthesis	(D)			Maria
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.27	0.28	0.37	0.31	0.30	0.36	-	0.27
Rainy season	0.25	0.26	0.28	0.29	0.30	0.32	0.32	0.29
Mean	0.27	0.27	0.31	0.29	0.29	0.33	0.16	0.28
Kerala local								
Summer season	0.32	0.32	0.33	0.38	0.38	0.43	-	0.31
Rainy season	0.23	0.24	0.28	0.31	0.33	0.32	0.46	0.31
Mean	0.27	0.28	0.30	0.34	0.36	0.37	0.23	0.31
Summer season mean	0.29	0.30	0.35	0.35	0.34	0.39	-	0.29
Rainy season mean	0.24	0.25	0.29	0.30	0.31	0.31	0.39	0.30
Grand mean	0.27	0.27	0.30	0.32	0.32	0.35	0.19	
				•				
	D	V	S	D x V	D x S	V x S	D x V x S	
SEd	0.006	0.004	0.004	0.009	0.009	0.005	0.013	
CD (P=0.05)	0.02	0.007	0.007	0.02	0.02	0.01	0.03	

Table 27. Area (cm<sup>2</sup>) of seeds as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala local raised during seed growth and development in summer and rainy seasons

Verioty (V) /cooper (C)			Days	after anthesis (I	D)			Maan
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.52	0.53	0.54	0.56	0.63	0.74	-	0.51
Rainy season	0.34	0.55	0.58	0.56	0.64	0.63	0.63	0.56
Mean	0.45	0.53	0.55	0.55	0.64	0.68	0.31	0.53
Kerala local								
Summer season	0.64	0.64	0.71	0.86	0.89	0.90	-	0.66
Rainy season	0.49	0.50	0.55	0.71	0.71	0.67	0.94	0.66
Mean	0.59	0.57	0.60	0.78	0.79	0.79	0.47	0.66
Summer season mean	0.57	0.60	0.62	0.70	0.76	0.82	-	0.59
Rainy season mean	0.44	0.53	0.54	0.64	0.67	0.65	0.78	0.60
Grand mean	0.52	0.55	0.58	0.67	0.71	0.74	0.39	
							•	
	D	V	S	D x V	D x S	VxS	D x V x S	
SEd	0.01	0.005	0.005	0.02	0.02	0.01	0.02	
CD (P=0.05)	0.02	0.01	0.01	0.03	0.03	0.02	0.04	

Table 28. Perimeter (cm) of embryo as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala Local raised during seed growth and development in summer and rainy seasons

Variety (V) /seesen (C)			Days a	after anthesis (I	<b>D</b> )			Mean
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	2.21	2.22	2.61	2.36	2.34	2.5	-	2.03
Rainy season	2.38	2.02	2.08	2.14	2.22	2.25	2.36	2.21
Mean	2.30	2.12	2.35	2.25	2.28	2.37	1.18	2.10
Kerala local								
Summer season	2.32	2.10	2.05	2.40	2.51	2.38	2.92	2.18
Rainy season	2.60	2.50	2.40	2.52	2.52	2.80	-	2.38
Mean	2.40	2.29	2.23	2.46	2.51	2.57	1.46	2.30
Summer season mean	2.38	2.35	2.51	2.44	2.43	2.63	-	2.10
Rainy season mean	2.35	2.06	2.07	2.27	2.36	2.32	2.64	2.30
Grand mean	2.20	2.30	2.35	2.37	2.40	2.47	1.32	
	D	V	S	D x V	D x S	VxS	D x V x S	
SEd	0.02	0.01	0.01	0.03	0.03	0.02	0.05	
CD (P=0.05)	0.05	0.03	0.03	0.06	0.07	NS	0.10	

Table 29. Perimeter (cm) of seeds with stages of maturity as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala local raised during seed growth and development in summer and rainy seasons

Variates (V) Jacques (C)			Day	s after anthesis	(D)			Mean
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	2.90	2.92	3.47	3.19	3.22	3.48	-	2.74
Rainy season	2.53	3.04	2.78	3.03	3.20	3.16	3.21	2.99
Mean	2.72	2.98	3.13	3.11	3.21	3.32	1.60	2.87
Kerala local								
Summer season	3.37	3.29	3.36	3.77	3.76	3.87	-	3.06
Rainy season	3.07	2.86	2.93	3.45	3.42	3.36	4.06	3.31
Mean	3.22	3.08	3.15	3.61	3.59	3.61	2.03	3.18
Summer season mean	3.14	3.11	3.42	3.48	3.49	3.67	-	2.90
Rainy season mean	2.80	2.95	2.86	3.24	3.31	3.26	3.63	3.15
Grand mean	2.97	3.03	3.14	3.36	3.40	3.47	1.81	
	D	V	S	D x V	D x S	V x S	DxVxS	
SEd	0.02	0.02	0.06	0.07	0.07	0.03	0.09	
CD (P=0.05)	0.09	0.05	0.05	0.13	0.13	NS	0.18	

Table 30. Length (cm) of embryo with different stages of maturity as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variety (V) /seesen (C)			Day	ys after anthesis (	D)			Mean
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.88	0.86	1.04	0.91	0.90	0.98	-	0.80
Rainy season	0.87	0.77	0.78	0.83	0.87	0.86	0.93	0.85
Mean	0.88	0.81	0.91	0.88	0.88	0.91	0.47	0.82
Kerala local								
Summer season	1.04	1.00	0.95	1.04	1.11	1.09	-	0.89
Rainy season	0.92	0.82	0.83	0.94	0.98	0.94	1.17	0.94
Mean	0.98	0.91	0.89	0.98	1.05	1.01	0.59	0.92
Summer season mean	0.96	0.93	0.99	0.98	1.00	1.04	-	0.84
Rainy season mean	0.90	0.79	0.80	0.88	0.93	0.89	1.05	0.89
Grand mean	0.93	0.86	0.90	0.93	0.97	0.97	0.53	
	D	V	S	D x V	D x S	VxS	D x V x S	
SEd	0.02	0.01	0.01	0.02	0.02	0.01	0.03	
CD (P=0.05)	0.03	0.02	0.02	0.04	0.04	NS	0.06	

Table 31. Length (cm) of seed with different stages of maturity as measured in Image Analysis System for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variates (V) /sacces (C)			Day	ys after anthesis	(D)			Maar
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	1.20	1.12	1.31	1.07	1.16	1.28	-	1.02
Rainy season	0.94	1.03	1.05	1.09	1.15	1.16	1.13	1.08
Mean	1.07	1.08	1.18	1.08	1.15	1.22	0.57	1.05
Kerala local								
Summer season	1.30	1.23	1.23	1.39	1.41	1.46	-	1.15
Rainy season	1.16	1.08	1.09	1.30	1.27	1.21	1.45	1.22
Mean	1.23	1.17	1.17	1.35	1.35	1.34	0.72	1.18
Summer season mean	1.25	1.18	1.27	1.23	1.29	1.37	-	1.08
Rainy season mean	1.04	1.06	1.08	1.20	1.21	1.19	1.29	1.15
Grand mean	1.15	1.12	1.17	1.22	1.25	1.27	0.65	
	D	V	S	D x V	D x S	V x S	DxVxS	
SEd	0.02	0.01	0.02	0.02	0.03	0.02	0.04	
CD (P=0.05)	0.03	0.01	0.02	0.05	0.05	NS	0.07	

Table 32. Width (cm) of embryo with different stages of maturity as measured in Image Analysis system for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variaty (V) /accord (C)			Day	s after anthesis (	(D)			Mean
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.43	0.46	0.49	0.48	0.47	0.51	-	0.40
Rainy season	0.44	0.47	0.47	0.47	0.46	0.47	0.48	0.46
Mean	0.43	0.46	0.48	0.48	0.47	0.49	0.24	0.44
Kerala local								
Summer season	0.46	0.44	0.48	0.51	0.51	0.55	-	0.42
Rainy season	0.37	0.37	0.39	0.46	0.47	0.46	0.53	0.43
Mean	0.41	0.40	0.43	0.48	0.49	0.50	0.27	0.43
Summer season mean	0.44	0.45	0.48	0.50	0.49	0.53	-	0.41
Rainy season mean	0.40	0.42	0.43	0.46	0.47	0.47	0.50	0.45
Grand mean	2.20	2.30	2.35	2.37	2.40	2.47	1.32	
	D	V	S	D x V	D x S	VxS	DxVxS	
SEd	0.01	0.03	0.01	0.01	0.01	0.005	0.02	
CD (P=0.05)	0.02	NS	0.01	0.02	0.02	0.01	0.04	

Table 33. Width (cm) of seeds with different stages of maturity as measured in Image Analysis system for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Mariata (M) /aaaaa (C)			Da	ys after anthesis	(D)			Maria
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.60	0.66	0.73	0.70	0.72	0.75	-	0.59
Rainy season	0.45	0.72	0.71	0.70	0.73	0.74	0.76	0.69
Mean	0.53	0.69	0.72	0.70	0.72	0.74	0.38	0.64
Kerala local								
Summer season	0.67	0.63	0.76	0.80	0.82	0.83	-	0.64
Rainy season	0.62	0.61	0.62	0.72	0.75	0.75	0.89	0.70
Mean	0.65	0.62	0.69	0.76	0.78	0.79	0.44	0.67
Summer season mean	0.64	0.65	0.74	0.75	0.77	0.79	-	0.62
Rainy season mean	0.54	0.66	0.67	0.71	0.74	0.74	0.82	0.70
Grand mean	0.59	0.65	0.70	0.73	0.75	0.76	0.41	
	D	V	S	D x V	D x S	V x S	DxVxS	
SEd	0.01	0.005	0.005	0.01	0.01	0.006	0.02	
CD (P=0.05)	0.02	0.01	0.01	0.03	0.02	0.01	0.03	

Table 34. Circularity of embryo with different stages of maturity as measured in Image Analysis system for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Mariatry (M) (account (C)			Day	s after anthesis	(D)			Maan
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.69	0.70	0.66	0.68	0.69	0.68	-	0.58
Rainy season	0.70	0.76	0.73	0.73	0.70	0.72	0.68	0.72
Mean	0.70	0.73	0.69	0.70	0.69	0.70	0.34	0.65
Kerala local								
Summer season	0.60	0.64	0.68	0.71	0.72	0.68	-	0.57
Rainy season	0.63	0.66	0.66	0.68	0.65	0.67	0.65	0.66
Mean	0.62	0.65	0.67	0.69	0.68	0.68	0.32	0.62
Summer season mean	0.65	0.67	0.67	0.69	0.70	0.67	-	0.58
Rainy season mean	0.67	0.71	0.69	0.70	0.68	0.67	0.67	0.69
Grand mean	0.66	0.69	0.68	0.70	0.69	0.68	0.33	
			•	•		•		
	D	V	S	D x V	D x S	V x S	D x V x S	
SEd	0.01	0.004	0.004	0.01	0.01	0.005	0.02	
CD (P=0.05)	0.02	0.01	0.01	0.02	0.02	0.01	0.03	

Table 35. Circularity of seeds with different stages of maturity as measured in Image Analysis System for ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variety (V)			Day	's after anthesis	(D)			Maar
/season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.66	0.75	0.74	0.77	0.76	0.74	-	0.63
Rainy season	0.68	0.82	0.81	0.79	0.78	0.77	0.77	0.63
Mean	0.67	0.78	0.78	0.78	0.77	0.76	0.39	0.70
Kerala local								
Summer season	0.70	0.71	0.75	0.75	0.76	0.73	-	0.63
Rainy season	0.72	0.73	0.73	0.73	0.73	0.76	0.74	0.73
Mean	0.71	0.72	0.74	0.74	0.75	0.75	0.37	0.68
Summer season mean	0.68	0.73	0.74	0.76	0.76	0.74	-	0.63
Rainy season mean	0.70	0.77	0.77	0.76	0.76	0.77	0.76	0.76
Grand mean	0.69	0.75	0.76	0.76	0.76	0.75	0.38	
	D	V	S	D x V	D x S	V x S	DxVxS	
SEd	0.01	0.003	0.003	0.01	0.01	0.005	0.01	
CD (P=0.05)	0.01	0.01	0.01	0.08	0.02	0.01	0.03	

Table 36. Elongation of embryo with different stages of maturity as measured in Image Analysis system for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variaty (V) /aaaan (C)			Day	s after anthesis (	(D)			Maan
Variety (V) /season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.46	0.54	0.48	0.51	0.50	0.50	-	0.43
Rainy season	0.52	0.64	0.58	0.58	0.54	0.55	0.52	0.56
Mean	0.49	0.59	0.53	0.54	0.52	0.53	0.26	0.49
Kerala local								
Summer season	0.41	0.44	0.50	0.53	0.53	0.50	-	0.42
Rainy season	0.44	0.46	0.48	0.50	0.49	0.49	0.48	0.48
Mean	0.43	0.45	0.49	0.51	0.51	0.49	0.23	0.45
Summer season mean	0.44	0.49	0.49	0.52	0.51	0.50	-	0.42
Rainy season mean	0.48	0.55	0.53	0.54	0.52	0.52	0.49	0.52
Grand mean	0.46	0.52	0.51	0.53	0.52	0.51	0.25	
	D	V	S	D x V	D x S	VxS	D x V x S	
SEd	0.01	0.005	0.005	0.02	0.02	0.01	0.20	
CD (P=0.05)	0.02	0.01	0.01	0.03	0.03	0.02	0.04	

Table 37. Elongation of seeds with different stages of maturity as measured in Image Analysis system for Ash gourd varieties CO 2 and Kerala local raised in summer and rainy seasons

Variety (V)			Days	after anthesis (D	0)			Maria
/season (S)	20	30	40	50	60	70	80	Mean
CO 2								
Summer season	0.51	0.53	0.52	0.70	0.62	0.62	-	0.50
Rainy season	0.58	0.70	0.70	0.66	0.64	0.63	0.66	0.66
Mean	0.54	0.62	0.62	0.68	0.63	0.63	0.33	0.58
Kerala local								
Summer season	0.52	0.55	0.59	0.60	0.59	0.60	-	0.49
Rainy season	0.55	0.57	0.57	0.56	0.58	0.60	0.61	0.58
Mean	0.54	0.56	0.58	0.58	0.59	0.60	0.30	0.54
Summer season mean	0.52	0.54	0.56	0.66	0.60	0.61	-	0.49
Rainy season mean	0.56	0.64	0.64	0.61	0.61	0.62	0.62	0.62
Grand mean	0.54	0.59	0.59	0.63	0.61	0.62	0.32	
							•	
	D	V	S	D x V	D x S	V x S	D x V x S	
SEd	0.02	0.01	0.01	0.02	0.22	0.02	0.03	
CD (P=0.05)	0.03	0.02	0.02	NS	0.05	0.03	0.62	

Table 43. Field emergence (%) of seeds extracted from the peduncular (P), intermediate (I) and stylar (S) segments of bottom, middle and top fruits harvested during development and maturation of ash gourd cv. CO 2 in rainy season

					Node position of fruit (N)							Segment Mean				
Days after		Botto	om (B)			Midd	le (M)			Тор	(T)					Grand
anthesis (D)						Fruit se	gments (S	S)					P	I	S	Mean
	P	I	S	Mean	P	I	S	Mean	P	I	S	Mean				
40	34.5	42.6	47.5	41.5	34.5	42.6	47.5	41.5	32.0	33.0	55.5	40.2	33.6	39.4	50.1	41.0
50	83.2	85.6	86.2	85.0	82.3	85.6	86.2	84.7	74.5	87.0	88.5	83.3	80.0	86.0	86.9	84.3
60	85.2	86.2	87.2	86.2	84.3	86.2	87.2	85.9	81.5	89.0	90.0	86.8	83.6	87.1	88.1	86.3
70	87.6	88.0	89.0	88.2	85.6	87.5	89.0	87.4	89.0	91.2	90.1	90.1	87.4	88.9	89.4	88.6
Mean	72.6	75.6	77.5	75.2	71.7	75.5	77.5	74.9	69.3	75.0	81.0	75.1	71.2	75.4	78.6	
				D	N	S	D x N	D x S	NxS	x S D x N x S						
SEd				0.37	0.29	0.28	0.64	0.64	64 NS 1.10							
CD (P=0.0	)5)			0.73	0.57	0.57	1.27	1.27		2.:	20					

Table 41. Dry weight (g) of seeds extracted from the peduncular (P), intermediate (I) and stylar (S) segments of bottom, middle and top fruits harvested during development and maturation of ash gourd cv. CO 2 in rainy season

				•	No	de positi	on of fru	it (N)		-			Seg	gment M	ean	
Days		Botto	om (B)			Midd	le (M)			Тор	(T)					Grand
after anthesis						Fruit seg	gments (S	S)					P	I	S	Mean
(D)	P	I	S	Mean	P	I	S	Mean	P	I	S	Mean				
30	28.2	31.4	30.2	29.9	22.1	27.0	25.2	24.7	20.4	26.1	25.1	23.9	23.6	28.1	26.8	26.2
40	33.0	36.6	35.1	34.9	32.1	34.0	34.0	33.4	31.0	38.0	36.1	35.0	32.0	36.2	35.0	34.4
50	35.2	39.2	38.0	37.5	33.7	36.0	38.0	35.9	35.0	39.1	38.0	37.4	34.6	38.1	38.0	36.9
60	35.2	39.5	37.6	37.4	35.0	37.5	38.2	36.9	37.2	40.1	39.6	38.9	35.8	39.0	38.4	37.7
70	36.0	39.0	37.0	37.3	36.0	37.0	38.0	37.0	39.0	41.4	41.0	40.5	37.0	39.1	38.7	38.3
Mean	26.5	37.1	35.6	35.4	31.8	34.3	34.7	33.6	32.5	36.9	35.9	35.1	30.2	36.1	35.4	
				D	N	S	D x N	D x S	S NxS DxNxS				•	•		
SEd				0.32	0.25	0.25	0.56	0.56	0.43	0.9	97					
CD (P=0.0	0.05) 0.64 0.49 0.49 1.11 1.11 0.87 1.93															

Table 40. Moisture content (%) of seeds extracted from the peduncular (P), intermediate (I) and stylar (S) segments of bottom, middle and top fruits harvested during development and maturation of ash gourd cv. CO 2 in rainy season

					No	de positi	on of fru	it (N)					Segment Mean			
Days after		Botto	om (B)			Midd	le (M)			Тор	(T)					Grand
anthesis (D)						Fruit se	gments (S	S)					P	I	S	Mean
	P	I	S	Mean	P	I	S	Mean	P	I	S	Mean				
30	39.5	30.7	28.1	32.8	60.5	59.0	56.4	58.6	69.8	65.3	62.3	65.8	56.6	51.7	48.9	52.4
40	30.5	27.8	25.8	28.0	43.2	42.2	41.0	42.1	48.4	36.7	33.2	39.4	40.7	33.3	33.3	36.5
50	30.1	27.0	26.0	27.7	41.2	40.0	39.7	40.3	40.1	34.6	34.2	36.3	37.1	33.9	33.3	34.8
60	31.7	30.0	30.0	30.6	41.2	40.0	39.8	40.3	36.8	31.3	30.2	32.8	36.6	33.7	33.3	34.6
70	31.7	31.0	30.0	30.9	40.1	39.2	39.2	39.5	32.1	30.1	30.1	30.8	34.6	33.4	33.1	33.7
Mean	32.7	29.3	28.0	30.0	45.2	44.0	43.2	44.1	45.4	39.6	38.0	41.0	41.1	37.2	36.4	
				D	N	S	DxN	D x S	NxS	Ux S Dx Nx S						
SEd				0.29	0.22	0.22	0.50	0.50	0.39 0.87							
CD (P=0.0	)5)			0.57	0.44	0.44	0.99	0.99	0.77	1.	72					

Table 42. Germination (%) of seeds extracted from the peduncular (P), intermediate (I) and stylar (S) segments of bottom, middle and top fruits harvested during development and maturation of ash gourd cv. CO 2 in rainy season

					No	de positi	on of fru	it (N)					Seg	gment Mo	ean	
Days		Botto	om (B)			Midd	le (M)			Тор	(T)					Grand
after anthesis						Fruit se	gments (S	S)					P	I	S	Mean
(D)	P	I	S	Mean	P	I	S	Mean	P	I	S	Mean				
40	36.0	46.5	51.1	44.5	35.2	45.2	49.2	43.2	32.0	33.0	55.5	40.1	34.4	41.6	52.0	42.6
50	85.1	88.2	88.9	87.4	80.2	86.0	88.0	84.7	89.5	89.0	89.5	89.3	84.9	87.7	88.8	87.1
60	85.2	88.0	90.0	87.7	87.0	88.5	89.5	88.3	90.1	91.0	90.0	90.4	87.4	89.2	89.8	88.8
70	87.6	89.0	90.0	88.9	87.1	89.0	89.5	88.5	90.5	91.2	92.2	91.3	88.4	89.7	90.6	89.6
Mean	73.5	77.9	80.0	77.1	72.4	77.1	79.0	76.1	75.7	76.0	81.7	77.8	73.8	77.0	80.3	77.0
				D	N	S	D x N	D x S	NxS	x S D x N x S						
SEd				0.13	0.10	0.10	0.24	0.24	0.18 0.41							
CD (P=0.0	=0.05) 0.27 0.20 0.20 0.47 0.47 0.36 0.81															

Table 44. Dry weight of seedlings (mg seedlings<sup>-10</sup>) of seeds extracted from the peduncular (P), intermediate (I) and stylar (S) segments of bottom, middle and top fruits harvested during development and maturation of ash gourd cv. CO 2 in rainy season

					No	de positi	on of fru	it (N)					Seg	gment M	ean	
Days after		Botto	om (B)			Midd	le (M)			Top	(T)					Grand
anthesis (D)						Fruit se	gments (S	S)					P	I	S	Mean
	P	I	S	Mean	P	I	S	Mean	P	I	S	Mean				
40	20.2	24.5	27.6	24.1	20.0	24.2	26.3	23.5	20.1	23.8	23.0	22.3	20.1	24.1	25.6	23.3
50	31.6	33.1	35.3	33.3	30.2	34.1	35.3	33.2	30.6	35.8	34.7	33.7	30.8	34.3	35.1	33.4
60	34.5	35.8	39.0	36.4	32.2	35.9	35.9	34.7	37.1	38.5	38.4	38.0	34.6	36.7	37.6	36.4
70	38.1	39.6	39.5	39.0	36.5	37.8	39.0	37.8	37.8	40.1	41.2	39.7	37.5	39.1	39.9	38.8
Mean	31.1	33.3	35.4	33.2	29.8	33.0	34.1	32.3	31.4	34.5	34.3	33.4	30.8	33.5	34.5	
				D	N	S	D x N	D x S	NxS	X S D x N x S						
SEd				0.07	0.06	0.06	0.13	0.13	0.09 0.22							
CD (P=0.0	=0.05) 0.14 0.11 0.11 0.25 0.25 0.19 0.44															

Table 45. The effect of fruit storage under ambient condition on fruit weight loss and per cent loss in ash gourd varieties, CO 2 and Kerala local

1	Kerala local	Weight	loss (%)				
Period of	C	Fruit weigh O 2	, 0	la local	<u> </u>	` /	
Storage (Months)	Initial	At extraction	Initial	At extraction	CO 2	Kerala Local	Mean
0	5.00	5.00	5.10	5.10	0	0	0
1	4.60	4.45	4.58	4.44	3.7 (11.0)	2.51 (9.1)	3.1 (10.0)
2	5.67	5.30	5.76	5.33	7.8 (16.1)	7.5 (15.8)	7.60 (16.0)
3	4.50	4.00	4.46	4.05	7.6 (15.9)	9.2 (17.6)	8.4 (16.8)
4	4.70	3.93	4.68	3.93	12.8 (20.9)	16.0 (23.6)	14.4 (22.3)
5	4.85	4.03	4.82	4.03	13.1 (21.3)	16.4 (23.9)	14.8 (22.6)
6	4.90	4.04	4.86	4.04	15.6 (23.3)	16.8 (24.2)	16.2 (23.7)
7	4.80	3.52	4.87	3.52	27.2 (31.5)	27.7 (31.7)	27.5 (31.6)
8	5.10	3.75	5.13	3.75	31.5 (34.1)	26.9 (31.2)	29.1 (32.7)
9	4.65	3.14	4.61	3.14	34.5 (36.0)	31.8 (34.3)	33.1 (35.1)
10	5.00	3.24	4.59	3.24	30.2 (33.4)	29.4 (32.8)	29.8 (33.1)
11	4.95	2.92	4.93	2.92	45.5 (42.4)	40.8 (39.7)	43.1 (41.0)
12	4.90	2.81	4.41	2.21	52.9 (46.7)	49.9 (44.9)	51.4 (45.8)
Mean	4.90	4.30	4.83	3.8	21.8 (25.9)	21.1 (25.6)	

	P	V	P x V
SEd	0.03	0.01	0.04
CD (P=0.05%)	0.05	0.02	0.07

Table 46. The effect of fruit storage under ambient condition on 100 seed weight and moisture content in ash gourd varieties CO 2 and Kerala local

Periods of	100	) seed weight	(g)	Mo	isture content	(%)
storage (Months)	CO 2	Kerala local	Mean	CO 2	Kerala local	Mean
0	5.62	9.62	7.62	57.9 (49.6)	54.4 (49.8)	58.2 (49.7)
1	5.86	9.62	7.74	54.0 (47.3)	57.5 (49.3)	55.8 (48.3)
2	5.92	9.71	7.81	43.3 (41.0)	54.1 (47.3)	48.7 (44.2)
3	6.22	9.01	7.61	41.5 (40.1)	51.4 (45.8)	46.5 (42.1)
4	6.32	9.60	7.96	40.8 (39.7)	50.8 (45.4)	45.8 (42.8)
5	5.82	9.62	7.72	38.8 (38.8)	50.0 (45.0)	44.4 (41.9)
6	5.42	9.50	7.46	39.5 (38.9)	50.0 (45.0)	44.8 (41.9)
7	5.32	9.60	7.46	34.9 (36.2)	50.0 (45.0)	42.5 (40.6)
8	5.30	8.92	7.11	34.8 (36.1)	47.4 (43.5)	41.1 (39.8)
9	5.43	8.34	6.90	33.6 (35.4)	47.7 (43.6)	40.6 (39.5)
10	5.12	8.32	6.72	31.4 (34.0)	49.3 (44.6)	40.3 (39.3)
11	5.03	6.75	5.89	31.1 (33.9)	39.9 (39.2)	35.5 (36.5)
12	5.00	6.15	5.60	31.0 (33.9)	37.7 (39.2)	34.4 (36.5)
Mean	5.60	8.8		39.5 (38.8)	49.6 (44.7)	44.6 (41.8)
	Р	V	PxV	P	V	PxV
SEd	0.23	0.10	0.33	0.03	0.20	0.05
CD (P=0.05)	0.50	0.20	0.70	0.06	0.03	0.10

Table 47. The effect of fruit storage under ambient condition on fresh weight and dry weight of seed fruit<sup>-1</sup> in ash gourd varieties CO 2 and Kerala local

Periods of	Fresh	weight of see	ed (g)	Dry weight of seed (g)		
storage (Months)	CO 2	Kerala local	Mean	CO 2	Kerala local	Mean
0	81.3	180.3	130.8	50.6	125.0	87.8
1	82.3	182.4	132.3	52.7	125.8	89.3
2	84.0	182.8	133.4	53.3	126.2	89.8
3	80.3	183.3	131.8	55.9	117.1	86.5
4	80.1	183.7	131.9	56.9	124.8	90.9
5	78.4	184.8	131.6	52.4	125.0	88.7
6	70.4	182.8	126.6	48.8	123.5	86.0
7	70.1	180.3	125.2	47.9	124.8	86.4
8	69.5	166.4	117.9	47.7	115.9	81.8
9	70.4	167.8	119.1	48.9	108.4	78.7
10	70.0	166.7	118.4	46.0	108.2	77.1
11	64.9	167.6	116.3	47.3	110.3	78.8
12	62.3	162.3	112.3	47.9	111.4	79.7
Mean	74.1	172.2		50.5	118.9	

	P	V	P x V	P	V	P x V
SEd	0.04	0.02	0.06	0.07	0.03	0.10
CD (P=0.05)	0.09	0.03	0.12	0.13	0.05	0.20

Table 48. The effect of fruit storage under ambient condition on speed of germination and germination in ash gourd varieties CO 2 and Kerala local

Periods of	Spe	ed of germina	tion		Germination (%	6)
storage (Months)	CO 2	Kerala local	Mean	CO 2	Kerala local	Mean
0	10.1	3.2	6.7	36.5 (37.2)	37.8 (37.2)	37.2 (37.2)
1	10.9	4.6	7.8	45.6 (42.4)	45.5 (42.4)	45.6 (42.4)
2	12.1	5.7	8.9	65.5	57.8	61.7
3	12.5	9.2	10.9	(54.0) 80.5	(47.2) 75.2	(50.6) 77.9
4	12.8	11.3	12.0	(63.8) 83.5	(70.1) 83.5	66.9 83.5
5	13.1	11.8	12.5	(66.0) 88.5	(66.0) 87.5	(66.0)
6	13.9	11.8	12.9	(70.1) 88.5	(70.0) 89.5	(70.5) 89.0
7	14.4	11.6	13.0	(70.1) 90.5	(71.0) 91.5	(70.7) 91.0
8	14.5	11.8	13.2	(72.0) 94.5	(72.5) 91.5	(72.0) 92.9
9	14.0	13.2	13.6	(76.4) 89.5	90.2	(74.5) 89.6
10	13.8	12.6	13.2	(71.0) 89.5	(70.9) 89.7	(70.9) 89.6
11	12.7	11.2	12.0	(71.0) 83.5	(71.0) 87.9	(71.0) 85.7
12	11.3	10.1	10.7	(66.0) 80.5	(71.0) 86.6	(68.5) 83.6
Mean	12.6	9.8		(63.8) 78.2	(70.5) 78.0	(67.0)
Mean				(63.4)	(64.0)	
	P	V	P x V	P	V	P x V
SEd CD (P=0.05)	0.01	0.06 0.12	0.02	0.050	0.20	0.70 1.50

CD (P=0.05) 0.03 0.12 0.04 1.00 1.50 0.40

Table 49. The effect of fruit storage under ambient condition on shoot length and root length in ash gourd varieties CO 2 and Kerala local

Periods of storage	Sł	noot length (ci	n)	Root length (cm)		
(Months)	CO 2	Kerala local	Mean	CO 2	Kerala local	Mean
0	11.3	11.1	11.2	10.8	12.0	11.4
1	13.8	13.6	13.7	11.9	12.4	12.1
2	14.4	14.8	14.6	13.1	12.1	12.6
3	14.2	16.5	15.3	13.4	14.3	13.9
4	16.7	20.1	18.4	13.8	16.7	15.3
5	17.0	19.1	18.0	14.2	18.2	16.2
6	18.3	20.6	19.5	15.3	18.0	16.7
7	16.7	21.6	19.1	14.2	19.2	16.7
8	16.2	20.5	18.1	14.0	19.6	16.8
9	16.0	20.6	18.3	9.3	19.0	14.2
10	14.2	19.3	16.8	8.9	17.6	13.3
11	14.6	18.2	16.4	8.2	16.2	12.2
12	14.1	18.5	16.3	8.0	14.1	11.0
Mean	14.1	18.0	16.6	11.2	16.2	

	P	V	P x V	P	V	P x V
SEd	0.02	0.08	0.03	0.03	0.01	0.04
CD (P=0.05)	0.05	0.16	0.06	0.06	0.02	0.08

Table 50. The effect of fruit storage under ambient condition on dry weight of seedling and vigour index in ash gourd varieties CO 2 and Kerala local

Periods of storage (Months)	Dry weight of seedling (mg 10 seedling <sup>-1</sup> )			Vigour index		
(Wolldis)	CO 2	Kerala local	Mean	CO 2	Kerala local	Mean
0	27.3	30.1	28.7	807	873	840
1	30.3	31.3	30.8	1149	1183	1166
2	35.1	34.9	35.0	1801	1555	1678
3	37.1	39.0	38.0	2222	2316	2269
4	37.0	41.0	39.0	2546	3073	2809
5	38.5	40.6	39.6	2805	3264	3034
6	38.2	42.9	40.6	2957	3455	3206
7	35.0	42.5	38.8	2796	3733	3264
8	34.6	41.8	38.2	2854	3669	3261
9	34.7	39.1	36.9	2264	3572	2918
10	30.3	38.2	34.3	2067	3310	2688
11	32.0	35.0	33.5	1904	3024	2464
12	32.0	33.2	32.6	1779	2823	2301
Mean	34.0	37.7		2150	2758	

	P	V	PxV	P	V	P x V
SEd	0.03	0.01	0.05	0.74	0.30	1.05
CD (P=0.05)	0.06	0.02	0.10	1.52	0.60	2.16

Table 59. Cost of seed extraction by closed fermentation method in ash gourd cv. CO2 incurred during On Farm Trial at seed producers unit, Kondamuthunur, Sathyamangalam taluk, Erode district

S. No	Particulars	No. of Men/women	Man days	Rate per day (Rs)	Total cost (Rs)
1	Fruit cutting	8 men	5	60	2,400.00
2	Separating seed cavity along with adhering pulp, filling seed + pulp + juice contents in the plastic drums and kept for closed fermentation for 72 h	25 women	5	35	4375.00
3	Washing for 15 min. in running water	25 women in batches	5	35	4375.00
4	Initial drying at room temperature for 72 h and subsequent sun drying for 12 h	33 women	5	35	5775.00
5	Processing and packing of seeds	10 women	1	35	350.00
Т	17,275				

Table 60. Economics of seed extraction by closed fermentation method (Modified method)

S.No.	Particulars	
1.	Area	2 acres
2.	Variety	CO 2
3.	Total fruit yield	60,100 kg/ 2 acres
4.	Weight of good fruits after sorting	45,000 kg
5.	Rejected fruits for vegetable purpose	15,100 kg
6.	Seed extraction by modified method	
	a. Seed yield	305 kg / 2 acres
	b. Seed recovery	0.68 per cent
	c. Cost of seed extraction for 45,000 kg of fruits	Rs. 17,275.00
	d. Cost of seed extraction for one kg of fruits	Rs. 0.38
	e. Cost of extraction for one kg of seeds	Rs. 56.00
7.	Seed extraction by conventional method	
	a. Cost of seed extraction for 45,000 kg of fruits	Rs. 12,275.00
	b. Cost of seed extraction for one kg of fruits	Rs. 0.27
	c. Cost of extraction for one kg of seeds	Rs. 40.00
8.	Additional cost incurred for present modified method for extracting one kg of seeds	Rs. 16.00
9.	Cost of cultivation	
	a. Cultivation expenses	Rs. 20,000.00 / 2 acres
	b. Cost of seed extraction by modified method	Rs. 17,275.00 / 2 acres
	Total	Rs. 37,275.00 / 2 acres
10.	Gross income	
	a. Income from sale of seeds (305 kgs @ Rs. 400 /kg of seed)	Rs. 1,22,000.00 / 2 acres
	b. Income from sale of vegetables (15,100 kgs @ Rs. 2.00 /kg of fruit)	Rs. 30,200.00 / 2 acres
	Total	Rs. 1,52,200.00 / 2 acres

11.	Net income	
	a. For two acres	Rs. 1,14,925.00 / 2 acres
	b. For one acre	Rs. 57,462.00 / 1 acre
12.	Cost benefit ratio	1:3.1

Table 52. Effect of different fermentation, separation, washing and drying methods during storage on seed germination (%) of ash gourd cv. CO2

E(E)	Separation, washing and drying (W) methods	Fruit storage in months (P)					Mean
Fermentation (F) methods		1	2	3	4	5	
No fermentation	Sun drying (SD)	50.0 (45.0)	65.3 (53.9)	65.0(53.7)	82.0 (64.9)	81.3 (64.4)	68.7 (56.4)
	Dying for72h + SD	51.0(45.6)	84.3(66.7)	83.3(65.9)	84.6(66.9)	87.3(69.1)	78.1(62.9)
	Washing 15min + SD	69.7(56.6)	84.0(66.4)	91.3(73.0)	82.7(65.4)	87.7(69.4)	83.0(66.1)
	Washing 15 min. + Drying at RT for 72h + SD	73.0(58.7)	85.3(67.5)	87.0(68.9)	85.0(67.2)	88.3(70.0)	83.7(66.4)
	Mean	60.9(51.5)	79.8(63.7)	81.7(65.4)	83.6(66.1)	86.1(68.3)	78.4(62.9)
Closed fermentation	Sun drying (SD)	86.3(68.3)	82.7(65.4)	85.0(67.2)	87.7(69.4)	87.0(68.9)	85.7(67.9)
	Dying at room temperature (RT) for 72h + SD	85.3(67.8)	84.0(66.4)	83.7(66.2)	90.3(72.1)	94.0(75.9)	87.5(69.7)
	Washing 15 min + SD	85.0(67.2)	93.0(74.7)	93.7(75.6)	93.0(74.9)	92.0(74.3)	91.5(73.3)
	Washing 15min + Drying 72h + SD	93.3(75.1)	95.7(78.0)	95.0(77.1)	94.7(76.1)	99.0(85.4)	95.5(78.5)
	Mean	87.5(69.6)	88.8(71.5)	89.3(71.5)	91.4(73.3)	93.1(76.1)	90.0(72.3)
Open fermentation	Sun drying (SD)	68.3(55.8)	94.3(76.2)	88.0(69.7)	83.3(65.9)	95.3(77.5)	85.9(69.0)
	Dying at room temperature (RT) for 72h + SD	75.3(60.2)	82.3(65.1)	92.7(74.3)	93.0(74.7)	93.3(75.0)	87.3(69.9)
	Washing 15min + SD	78.3(62.3)	96.7(79.6)	86.0(68.0)	94.3(76.2)	93.7(75.6)	89.8(72.4)
	Washing 15min + Drying 72h + SD	87.7(69.1)	97.7(61.8)	93.7(75.5)	97.0(80.6)	95.7(78.3)	90.2(73.0)
	Mean	77.3(61.9)	87.8(72.7)	90.0(71.9)	91.9(74.4)	94.5(76.6)	88.3(71.0)
Separation, washing and drying mean	Sun drying (SD)	72.8(59.4)	78.6(62.9)	83.3(59.3)	87.8(70.0)	88.1(70.5)	80.1(64.4)
	Dying for72h + SD	75.9(62.1)	86.7(68.9)	81.7(64.9)	87.0(69.4)	90.3(72.1)	84.3(67.5)
	Washing 15min + SD	82.4(65.3)	85.9(69.5)	90.1(71.9)	91.4(73.3)	90.7(73.1)	88.1(70.6)
	Washing 15min + Drying 72h + SD	87.2(71.5)	88.3(72.1)	88.7(70.6)	92.1(74.1)	92.9(75.0)	89.8(72.7)
	Grand Mean	80.7(65.8)	81.5(65.0)	86.5(69.5)	88.1(70.9)	90.7(72.7)	-

	F	W	P	FxW	FxP	WxP	FxWxP
SEd	0.36	0.41	0.46	0.71	0.79	0.92	1.59
CD (P=0.05)	0.70	0.81	0.91	1.41	1.58	1.82	3.16

Table 51. Effect of different fermentation, separation, washing and drying methods during storage on seed speed of germination of ash gourd cv. CO 2

Formantation (E) mathada	Separation, washing and drying (W) methods	Fruit storage in months (P)					Mean
Fermentation (F) methods		1	2	3	4	5	
No fermentation	Sun drying (SD)	6.4	7.4	6.5	6.4	9.9	7.3
	Dying at room temperature (RT) for 72h + SD	6.4	9.1	5.2	8.5	10.9	8.0
	Washing 15min + SD	7.5	8.7	6.7	10.8	10.8	8.9
	Washing 15 min. + Drying at RT for 72h + SD	9.7	9.4	6.5	11.7	11.0	9.6
	Mean	7.5	8.6	9.2	9.4	10.7	8.5
Closed fermentation	Sun drying (SD)	11.6	11.7	12.2	14.4	13.7	12.7
	Dying at room temperature (RT) for 72h + SD	12.2	12.1	12.4	12.9	15.3	13.0
	Washing 15min + SD	11.5	12.8	13.8	14.7	13.7	13.3
	Washing 15 min. + Drying at RT for 72h + SD	14.1	13.0	13.3	13.4	14.4	13.7
	Mean	12.4	12.4	12.9	13.9	14.3	13.2
Open fermentation	Sun drying (SD)	9.4	11.2	12.0	13.8	14.4	12.2
	Dying at room temperature (RT) for 72h + SD	10.7	10.8	12.8	14.1	14.9	12.6
	Washing 15min + SD	11.5	11.1	12.7	13.5	14.0	12.6
	Washing 15 min. + Drying at RT for 72h + SD	12.3	13.8	13.1	11.3	15.1	13.1
	Mean	11.0	11.7	12.7	13.2	14.6	12.6

Separation, washing and drying mean	Sun drying (SD)	9.2	10.0	11.4	11.5	11.6	10.8
	Dying at room temperature (RT) for 72h + SD	9.7	10.7	10.8	12.0	12.9	11.2
	Washing 15min + SD	10.5	10.6	11.6	12.4	12.8	11.6
	Washing 15 min. + Drying at RT for 72h + SD	10.4	11.6	12.4	12.5	13.8	12.1
	Grand Mean	10.3	10.9	11.1	12.1	12.8	

	F	$\mathbf{W}$	P	F x W	FxP	WxP	FxWxP
SEd	0.05	0.06	0.07	0.10	0.12	0.14	0.24
CD (P=0.05)	0.10	0.12	0.14	0.21	0.24	0.27	0.47

Table 54. Effect of different fermentation, separation, washing and drying methods during storage on seed root length (cm) of ash gourd cv. CO 2

Formantation (F) mathods	Separation, washing and drying (W) methods		Frui	t storage in month	s (P)		Mean
Fermentation (F) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	10.2	9.7	10.4	11.3	10.5	10.4
	Dying at room temperature (RT) for 72h + SD	9.2	11.1	10.1	10.8	11.2	10.5
	Washing 15 min. +SD	9.7	9.9	10.5	11.4	11.7	10.6
	Washing 15 min. + Drying at RT for 72h + SD	11.1	9.8	10.9	11.8	10.9	10.9
	Mean	10.0	10.1	10.5	11.3	11.0	10.6
Closed fermentation	Sun drying (SD)	9.6	10.3	12.1	11.5	12.8	11.3
	Dying at room temperature (RT) for 72h + SD	10.9	11.4	11.9	12.3	12.6	11.8
	Washing 15 min. +SD	11.9	12.4	11.1	12.1	13.9	12.3
	Washing 15 min. + Drying at RT for 72h + SD	11.3	12.9	12.5	12.3	13.2	12.4
	Mean	10.9	11.7	11.9	12.0	13.1	11.9
Open fermentation	Sun drying (SD)	10.3	10.9	10.8	10.9	13.1	11.2
	Dying at room temperature (RT) for 72h + SD	9.9	11.5	10.2	10.9	12.0	10.9
	Washing 15 min. +SD	10.9	11.5	11.3	11.4	12.4	11.5
	Washing 15 min. + Drying at RT for 72h + SD	10.9	9.9	11.0	10.9	12.4	11.0
	Mean	10.5	10.9	10.9	11.0	12.5	11.1
Separation, washing and drying mean	Sun drying (SD)	10.0	10.9	10.6	10.9	12.4	10.9
	Dying at room temperature (RT) for 72h + SD	10.0	11.0	11.1	11.5	12.0	11.0
	Washing 15 min. +SD	10.4	10.9	11.2	11.7	12.5	11.3
	Washing 15 min. + Drying at RT for 72h + SD	11.2	11.3	11.5	11.8	12.5	11.6
	Grand Mean	10.5	10.9	11.1	11.2	12.3	-

	$\mathbf{F}$	W	P	FxW	FxP	WxP	FxWxP
SEd	0.02	0.02	0.02	0.03	0.03	0.03	0.06
CD (P=0.05)	0.03	0.03	0.03	0.05	0.06	0.07	0.12

Table 53. Effect of different fermentation and washing methods during storage on shoot length (cm) of seeds of Ash gourd cv. CO2

Formantation (F) mathada	Separation, washing and drying (W) methods		Fruit	storage in month	s (P)		Mean
Fermentation (F) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	12.0	10.5	12.7	14.1	16.2	13.1
	Dying at room temperature (RT) for 72h + SD	11.9	12.7	13.5	14.5	17.3	14.0
	Washing 15 min. +SD	13.6	13.3	13.9	15.4	17.8	14.8
	Washing 15 min. + Drying at RT for 72h + SD	11.5	13.2	13.4	15.3	17.2	14.1
	Mean	12.3	12.4	13.4	14.8	17.1	14.0
Closed fermentation	Sun drying (SD)	10.2	12.2	13.8	17.1	19.9	14.6
	Dying at room temperature (RT) for 72h + SD	11.5	11.8	13.6	18.7	20.4	15.2
	Washing 15 min. +SD	12.4	12.1	13.8	16.9	20.7	15.2
	Washing 15 min. + Drying at RT for 72h + SD	12.2	12.4	14.1	18.4	20.6	15.5
	Mean	11.6	12.1	13.8	17.8	20.4	15.1
Open fermentation	Sun drying (SD)	11.4	12.1	13.2	14.8	18.3	13.9
	Dying at room temperature (RT) for 72h + SD	11.8	12.5	13.0	14.4	17.5	13.8
	Washing 15 min. +SD	11.9	12.2	13.3	14.8	18.9	14.2
	Washing 15 min. + Drying at RT for 72h + SD	13.6	12.8	14.7	16.3	18.4	15.2
	Mean	12.1	12.4	13.5	15.0	18.3	14.3
Separation, washing and drying mean	Sun drying (SD)	11.2	11.6	13.3	15.3	18.1	13.9
	Dying at room temperature (RT) for 72h + SD	11.7	12.3	13.4	15.8	18.4	14.3
	Washing 15 min. +SD	12.5	12.6	13.6	15.7	19.1	14.7
	Washing 15 min. + Drying at RT for 72h + SD	12.5	12.8	14.0	16.6	18.9	14.9
	Grand Mean	12.3	13.6	13.6	15.9	18.6	-

	F	W	P	F x W	FxP	WxP	FxWxP
SEd	0.03	0.03	0.04	0.06	0.06	0.07	0.13
CD (P=0.05)	0.06	0.07	0.07	0.11	0.13	0.15	0.26

Table 55. Effect of different fermentation, separation, washing and drying methods during storage seed on seedling dry weight (mg/10<sup>-1</sup>) of ash gourd cv. CO 2

Formantation (E) matheda	Compaction weaking and draing (W) matheds		Fruit	t storage in months	(P)		Mean
Fermentation (F) methods	Separation, washing and drying (W) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	28.2	29.2	30.7	31.2	32.5	30.4
	Dying at room temperature (RT) for 72h + SD	29.4	31.5	32.3	33.7	34.6	32.3
	Washing 15 min. +SD	29.3	32.5	33.3	33.6	35.2	32.7
	Washing 15 min. + Drying at RT for 72h + SD	28.7	32.0	32.7	38.8	36.7	33.2
	Mean	28.9	31.3	32.3	33.6	34.7	32.2
Closed fermentation	Sun drying (SD)	33.7	38.9	38.4	29.2	41.1	38.2
	Dying at room temperature (RT) for 72h + SD	37.4	38.9	38.8	41.8	42.5	40.0
	Washing 15 min. +SD	38.4	38.9	38.8	43.2	43.1	40.5
	Washing 15 min. + Drying at RT for 72h + SD	39.0	38.6	41.8	45.1	44.4	41.8
	Mean	37.1	38.8	39.5	42.4	42.8	40.2
Open fermentation	Sun drying (SD)	36.4	36.7	39.0	40.6	41.0	38.0
	Dying at room temperature (RT) for 72h + SD	38.9	39.1	39.7	41.6	42.1	40.3
	Washing 15 min. +SD	38.8	39.3	40.0	41.8	42.1	40.4
	Washing 15 min. + Drying at RT for 72h + SD	39.6	41.3	42.8	44.2	44.2	42.4
	Mean	38.4	39.1	40.4	42.0	42.4	40.0
Separation, washing and drying mean	Sun drying (SD)	32.8	34.9	36.0	36.9	38.2	35.8
	Dying at room temperature (RT) for 72h + SD	35.2	36.5	36.9	39.0	39.7	37.5
	Washing 15 min. +SD	35.5	36.9	37.4	39.2	40.1	37.9
	Washing 15 min. + Drying at RT for 72h + SD	35.8	37.3	39.0	41.7	41.8	39.1
	Grand Mean	34.8	36.4	37.4	39.3	40.0	-

	$\mathbf{F}$	$\mathbf{W}$	P	F x W	FxP	WxP	FxWxP
SEd	0.17	0.19	0.21	0.33	0.37	0.43	0.75
CD (P=0.05)	0.33	0.38	0.43	0.66	0.74	0.86	1.48

Table 56. Effect of different fermentation, separation, washing and drying methods during storage on seed vigour index of ash gourd cv. CO 2

Formantation (F) mathada	Conception weaking and deving (W) matheda		Fruit s	storage in months	(P)		Mean
Fermentation (F) methods	Separation, washing and drying (W) methods	1	2	3	4	5	]
No fermentation	Sun drying (SD)	1321	1524	1368	1839	1997	1609
	Dying at room temperature (RT) for 72h + SD	1352	1914	1995	2081	2156	1899
	Washing 15 min. +SD	1921	2103	2285	1970	2273	2110
	Washing 15 min. + Drying at RT for 72h + SD	2105	1987	2105	1975	2308	2096
	Mean	1675	1882	1938	1966	2183	1921
Closed fermentation	Sun drying (SD)	1855	1956	2257	2511	2501	2216
	Dying at room temperature (RT) for 72h + SD	1941	2029	2399	2903	2616	2378
	Washing 15 min. +SD	2282	2230	2303	2756	2963	2507
	Washing 15 min. + Drying at RT for 72h + SD	2417	2343	2633	2903	3043	2668
	Mean	2124	2140	2398	2769	2781	2442
Open fermentation	Sun drying (SD)	1674	1714	2270	2433	2374	2093
	Dying at room temperature (RT) for 72h + SD	1815	2173	2016	2296	2547	2169
	Washing 15 min. +SD	1903	1987	2336	2415	2823	2273
	Washing 15 min. + Drying at RT for 72h + SD	2273	2289	2035	2641	2857	2419
	Mean	1917	2041	2140	2447	2650	2239
Separation, washing and drying mean	Sun drying (SD)	1646	1718	2066	2122	2313	1973
	Dying at room temperature (RT) for 72h + SD	1919	2037	2166	2172	2451	2149
	Washing 15 min. +SD	2170	2079	2186	2481	2569	2297
	Washing 15 min. + Drying at RT for 72h + SD	2201	2214	2271	2618	2668	2395
	Grand Mean	1979	2034	2168	2369	2466	-

	F	W	P	FxW	FxP	WxP	FxWxP
SEd	16.0	18.5	20.6	32.0	35.8	41.3	71.6
CD (P=0.05)	31.7	36.6	40.9	63.4	70.9	81.8	141.7

Table 52. Effect of different fermentation, separation, washing and drying methods during storage on seed germination (%) of ash gourd cv. CO2

Earmontation (E) mathada	Separation, washing and drying (W) methods		Fruit	storage in month	s (P)		Mean
Fermentation (F) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	50.0 (45.0)	65.3 (53.9)	65.0(53.7)	82.0 (64.9)	81.3 (64.4)	68.7 (56.4)
	Dying for72h + SD	51.0(45.6)	84.3(66.7)	83.3(65.9)	84.6(66.9)	87.3(69.1)	78.1(62.9)
	Washing 15min + SD	69.7(56.6)	84.0(66.4)	91.3(73.0)	82.7(65.4)	87.7(69.4)	83.0(66.1)
	Washing 15 min. + Drying at RT for 72h + SD	73.0(58.7)	85.3(67.5)	87.0(68.9)	85.0(67.2)	88.3(70.0)	83.7(66.4)
	Mean	60.9(51.5)	79.8(63.7)	81.7(65.4)	83.6(66.1)	86.1(68.3)	78.4(62.9)
Closed fermentation	Sun drying (SD)	86.3(68.3)	82.7(65.4)	85.0(67.2)	87.7(69.4)	87.0(68.9)	85.7(67.9)
	Dying at room temperature (RT) for 72h + SD	85.3(67.8)	84.0(66.4)	83.7(66.2)	90.3(72.1)	94.0(75.9)	87.5(69.7)
	Washing 15 min + SD	85.0(67.2)	93.0(74.7)	93.7(75.6)	93.0(74.9)	92.0(74.3)	91.5(73.3)
	Washing 15min + Drying 72h + SD	93.3(75.1)	95.7(78.0)	95.0(77.1)	94.7(76.1)	99.0(85.4)	95.5(78.5)
	Mean	87.5(69.6)	88.8(71.5)	89.3(71.5)	91.4(73.3)	93.1(76.1)	90.0(72.3)
Open fermentation	Sun drying (SD)	68.3(55.8)	94.3(76.2)	88.0(69.7)	83.3(65.9)	95.3(77.5)	85.9(69.0)
	Dying at room temperature (RT) for 72h + SD	75.3(60.2)	82.3(65.1)	92.7(74.3)	93.0(74.7)	93.3(75.0)	87.3(69.9)
	Washing 15min + SD	78.3(62.3)	96.7(79.6)	86.0(68.0)	94.3(76.2)	93.7(75.6)	89.8(72.4)
	Washing 15min + Drying 72h + SD	87.7(69.1)	97.7(61.8)	93.7(75.5)	97.0(80.6)	95.7(78.3)	90.2(73.0)
	Mean	77.3(61.9)	87.8(72.7)	90.0(71.9)	91.9(74.4)	94.5(76.6)	88.3(71.0)
Separation, washing and drying mean	Sun drying (SD)	72.8(59.4)	78.6(62.9)	83.3(59.3)	87.8(70.0)	88.1(70.5)	80.1(64.4)
	Dying for72h + SD	75.9(62.1)	86.7(68.9)	81.7(64.9)	87.0(69.4)	90.3(72.1)	84.3(67.5)
	Washing 15min + SD	82.4(65.3)	85.9(69.5)	90.1(71.9)	91.4(73.3)	90.7(73.1)	88.1(70.6)
	Washing 15min + Drying 72h + SD	87.2(71.5)	88.3(72.1)	88.7(70.6)	92.1(74.1)	92.9(75.0)	89.8(72.7)

Grand Mean		80.7(65.8)	81.5(65.0)	86.5(69.5)	88.1(70.9)	90.7(72.7)	-
	F	W	P	FxW	FxP	WxP	FxWxP
SEd	0.36	0.41	0.46	0.71	0.79	0.92	1.59
CD (P=0.05)	0.70	0.81	0.91	1.41	1.58	1.82	3.16

(Figures in parentheses are arc-sine transformed values)

Table 51. Effect of different fermentation, separation, washing and drying methods during storage on seed speed of germination of ash gourd cv. CO 2

Fermentation (F) methods	Separation, washing and drying (W) methods		Fruit	storage in month	ns (P)		Mean
rementation (r) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	6.4	7.4	6.5	6.4	9.9	7.3
	Dying at room temperature (RT) for 72h + SD	6.4	9.1	5.2	8.5	10.9	8.0
	Washing 15min + SD	7.5	8.7	6.7	10.8	10.8	8.9
	Washing 15 min. + Drying at RT for 72h + SD	9.7	9.4	6.5	11.7	11.0	9.6
	Mean	7.5	8.6	9.2	9.4	10.7	8.5
Closed fermentation	Sun drying (SD)	11.6	11.7	12.2	14.4	13.7	12.7
	Dying at room temperature (RT) for 72h + SD	12.2	12.1	12.4	12.9	15.3	13.0
	Washing 15min + SD	11.5	12.8	13.8	14.7	13.7	13.3
	Washing 15 min. + Drying at RT for 72h + SD	14.1	13.0	13.3	13.4	14.4	13.7
	Mean	12.4	12.4	12.9	13.9	14.3	13.2
Open fermentation	Sun drying (SD)	9.4	11.2	12.0	13.8	14.4	12.2
	Dying at room temperature (RT) for 72h + SD	10.7	10.8	12.8	14.1	14.9	12.6

	Washing 15min + SD Washing 15 min. + Drying at RT for 72h + SD	11.5 12.3	11.1 13.8	12.7 13.1	13.5 11.3	14.0 15.1	12.6 13.1
	Mean	11.0	11.7	12.7	13.2	14.6	12.6
Separation, washing and drying mean	Sun drying (SD)	9.2	10.0	11.4	11.5	11.6	10.8
	Dying at room temperature (RT) for 72h + SD	9.7	10.7	10.8	12.0	12.9	11.2
	Washing 15min + SD	10.5	10.6	11.6	12.4	12.8	11.6
	Washing 15 min. + Drying at RT for 72h + SD	10.4	11.6	12.4	12.5	13.8	12.1
	Grand Mean	10.3	10.9	11.1	12.1	12.8	

	${f F}$	W	P	FxW	FxP	WxP	FxWxP
SEd	0.05	0.06	0.07	0.10	0.12	0.14	0.24
CD (P=0.05)	0.10	0.12	0.14	0.21	0.24	0.27	0.47

Table 54. Effect of different fermentation, separation, washing and drying methods during storage on seed root length (cm) of ash gourd cv. CO 2

Formantation (E) mathada	Separation, washing and drying (W) methods		Frui	t storage in months	s (P)		Mean
Fermentation (F) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	10.2	9.7	10.4	11.3	10.5	10.4
	Dying at room temperature (RT) for 72h + SD	9.2	11.1	10.1	10.8	11.2	10.5
	Washing 15 min. +SD	9.7	9.9	10.5	11.4	11.7	10.6
	Washing 15 min. + Drying at RT for 72h + SD	11.1	9.8	10.9	11.8	10.9	10.9
	Mean	10.0	10.1	10.5	11.3	11.0	10.6
Closed fermentation	Sun drying (SD)	9.6	10.3	12.1	11.5	12.8	11.3
	Dying at room temperature (RT) for 72h + SD	10.9	11.4	11.9	12.3	12.6	11.8
	Washing 15 min. +SD	11.9	12.4	11.1	12.1	13.9	12.3
	Washing 15 min. + Drying at RT for 72h + SD	11.3	12.9	12.5	12.3	13.2	12.4
	Mean	10.9	11.7	11.9	12.0	13.1	11.9
Open fermentation	Sun drying (SD)	10.3	10.9	10.8	10.9	13.1	11.2
	Dying at room temperature (RT) for 72h + SD	9.9	11.5	10.2	10.9	12.0	10.9
	Washing 15 min. +SD	10.9	11.5	11.3	11.4	12.4	11.5
	Washing 15 min. + Drying at RT for 72h + SD	10.9	9.9	11.0	10.9	12.4	11.0
	Mean	10.5	10.9	10.9	11.0	12.5	11.1
Separation, washing and drying mean	Sun drying (SD)	10.0	10.9	10.6	10.9	12.4	10.9
	Dying at room temperature (RT) for 72h + SD	10.0	11.0	11.1	11.5	12.0	11.0
	Washing 15 min. +SD	10.4	10.9	11.2	11.7	12.5	11.3
	Washing 15 min. + Drying at RT for 72h + SD	11.2	11.3	11.5	11.8	12.5	11.6
	Grand Mean	10.5	10.9	11.1	11.2	12.3	-

	$\mathbf{F}$	W	P	FxW	FxP	WxP	FxWxP
SEd	0.02	0.02	0.02	0.03	0.03	0.03	0.06
CD (P=0.05)	0.03	0.03	0.03	0.05	0.06	0.07	0.12

Table 53. Effect of different fermentation and washing methods during storage on shoot length (cm) of seeds of Ash gourd cv. CO2

Formantation (F) mathada	Separation, washing and drying (W) methods		Fruit	storage in month	s (P)		Mean
Fermentation (F) methods	Separation, washing and drying (w) methods	1	2	3	4	5	
No fermentation	Sun drying (SD)	12.0	10.5	12.7	14.1	16.2	13.1
	Dying at room temperature (RT) for 72h + SD	11.9	12.7	13.5	14.5	17.3	14.0
	Washing 15 min. +SD	13.6	13.3	13.9	15.4	17.8	14.8
	Washing 15 min. + Drying at RT for 72h + SD	11.5	13.2	13.4	15.3	17.2	14.1
	Mean	12.3	12.4	13.4	14.8	17.1	14.0
Closed fermentation	Sun drying (SD)	10.2	12.2	13.8	17.1	19.9	14.6
	Dying at room temperature (RT) for 72h + SD	11.5	11.8	13.6	18.7	20.4	15.2
	Washing 15 min. +SD	12.4	12.1	13.8	16.9	20.7	15.2
	Washing 15 min. + Drying at RT for 72h + SD	12.2	12.4	14.1	18.4	20.6	15.5
	Mean	11.6	12.1	13.8	17.8	20.4	15.1
Open fermentation	Sun drying (SD)	11.4	12.1	13.2	14.8	18.3	13.9
	Dying at room temperature (RT) for 72h + SD	11.8	12.5	13.0	14.4	17.5	13.8
	Washing 15 min. +SD	11.9	12.2	13.3	14.8	18.9	14.2
	Washing 15 min. + Drying at RT for 72h + SD	13.6	12.8	14.7	16.3	18.4	15.2
	Mean	12.1	12.4	13.5	15.0	18.3	14.3
Separation, washing and drying mean	Sun drying (SD)	11.2	11.6	13.3	15.3	18.1	13.9
	Dying at room temperature (RT) for 72h + SD	11.7	12.3	13.4	15.8	18.4	14.3
	Washing 15 min. +SD	12.5	12.6	13.6	15.7	19.1	14.7
	Washing 15 min. + Drying at RT for 72h + SD	12.5	12.8	14.0	16.6	18.9	14.9
	Grand Mean	12.3	13.6	13.6	15.9	18.6	-

	F	W	P	F x W	FxP	WxP	FxWxP
SEd	0.03	0.03	0.04	0.06	0.06	0.07	0.13
CD (P=0.05)	0.06	0.07	0.07	0.11	0.13	0.15	0.26

Table 55. Effect of different fermentation, separation, washing and drying methods during storage seed on seedling dry weight (mg/10<sup>-1</sup>) of ash gourd cv. CO 2

Formantation (F) mathods	Separation, washing and drying (W) methods —		Fruit	t storage in months	(P)		Mean
Fermentation (F) methods		1	2	3	4	5	
No fermentation	Sun drying (SD)	28.2	29.2	30.7	31.2	32.5	30.4
	Dying at room temperature (RT) for 72h + SD	29.4	31.5	32.3	33.7	34.6	32.3
	Washing 15 min. +SD	29.3	32.5	33.3	33.6	35.2	32.7
	Washing 15 min. + Drying at RT for 72h + SD	28.7	32.0	32.7	38.8	36.7	33.2
	Mean	28.9	31.3	32.3	33.6	34.7	32.2
Closed fermentation	Sun drying (SD)	33.7	38.9	38.4	29.2	41.1	38.2
	Dying at room temperature (RT) for 72h + SD	37.4	38.9	38.8	41.8	42.5	40.0
	Washing 15 min. +SD	38.4	38.9	38.8	43.2	43.1	40.5
	Washing 15 min. + Drying at RT for 72h + SD	39.0	38.6	41.8	45.1	44.4	41.8
	Mean	37.1	38.8	39.5	42.4	42.8	40.2
Open fermentation	Sun drying (SD)	36.4	36.7	39.0	40.6	41.0	38.0
	Dying at room temperature (RT) for 72h + SD	38.9	39.1	39.7	41.6	42.1	40.3
	Washing 15 min. +SD	38.8	39.3	40.0	41.8	42.1	40.4
	Washing 15 min. + Drying at RT for 72h + SD	39.6	41.3	42.8	44.2	44.2	42.4
	Mean	38.4	39.1	40.4	42.0	42.4	40.0
Separation, washing and drying mean	Sun drying (SD)	32.8	34.9	36.0	36.9	38.2	35.8
	Dying at room temperature (RT) for 72h + SD	35.2	36.5	36.9	39.0	39.7	37.5
	Washing 15 min. +SD	35.5	36.9	37.4	39.2	40.1	37.9
	Washing 15 min. + Drying at RT for 72h + SD	35.8	37.3	39.0	41.7	41.8	39.1
	Grand Mean	34.8	36.4	37.4	39.3	40.0	-

	$\mathbf{F}$	$\mathbf{W}$	P	F x W	FxP	WxP	FxWxP
SEd	0.17	0.19	0.21	0.33	0.37	0.43	0.75
CD (P=0.05)	0.33	0.38	0.43	0.66	0.74	0.86	1.48

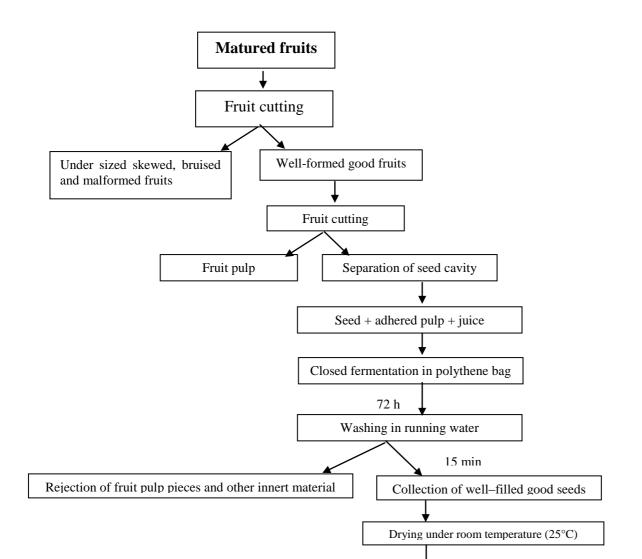
Table 56. Effect of different fermentation, separation, washing and drying methods during storage on seed vigour index of ash gourd cv. CO 2

Formantation (F) mathada	Conception weaking and deving (W) matheda		Fruit s	storage in months	(P)		Mean
Fermentation (F) methods	Separation, washing and drying (W) methods	1	2	3	4	5	]
No fermentation	Sun drying (SD)	1321	1524	1368	1839	1997	1609
	Dying at room temperature (RT) for 72h + SD	1352	1914	1995	2081	2156	1899
	Washing 15 min. +SD	1921	2103	2285	1970	2273	2110
	Washing 15 min. + Drying at RT for 72h + SD	2105	1987	2105	1975	2308	2096
	Mean	1675	1882	1938	1966	2183	1921
Closed fermentation	Sun drying (SD)	1855	1956	2257	2511	2501	2216
	Dying at room temperature (RT) for 72h + SD	1941	2029	2399	2903	2616	2378
	Washing 15 min. +SD	2282	2230	2303	2756	2963	2507
	Washing 15 min. + Drying at RT for 72h + SD	2417	2343	2633	2903	3043	2668
	Mean	2124	2140	2398	2769	2781	2442
Open fermentation	Sun drying (SD)	1674	1714	2270	2433	2374	2093
	Dying at room temperature (RT) for 72h + SD	1815	2173	2016	2296	2547	2169
	Washing 15 min. +SD	1903	1987	2336	2415	2823	2273
	Washing 15 min. + Drying at RT for 72h + SD	2273	2289	2035	2641	2857	2419
	Mean	1917	2041	2140	2447	2650	2239
Separation, washing and drying mean	Sun drying (SD)	1646	1718	2066	2122	2313	1973
	Dying at room temperature (RT) for 72h + SD	1919	2037	2166	2172	2451	2149
	Washing 15 min. +SD	2170	2079	2186	2481	2569	2297
	Washing 15 min. + Drying at RT for 72h + SD	2201	2214	2271	2618	2668	2395
	Grand Mean	1979	2034	2168	2369	2466	-

	F	W	P	FxW	FxP	WxP	FxWxP
SEd	16.0	18.5	20.6	32.0	35.8	41.3	71.6
CD (P=0.05)	31.7	36.6	40.9	63.4	70.9	81.8	141.7

FLOW CHART I

Step by step process to be followed for closed fermentation technique in ash gourd



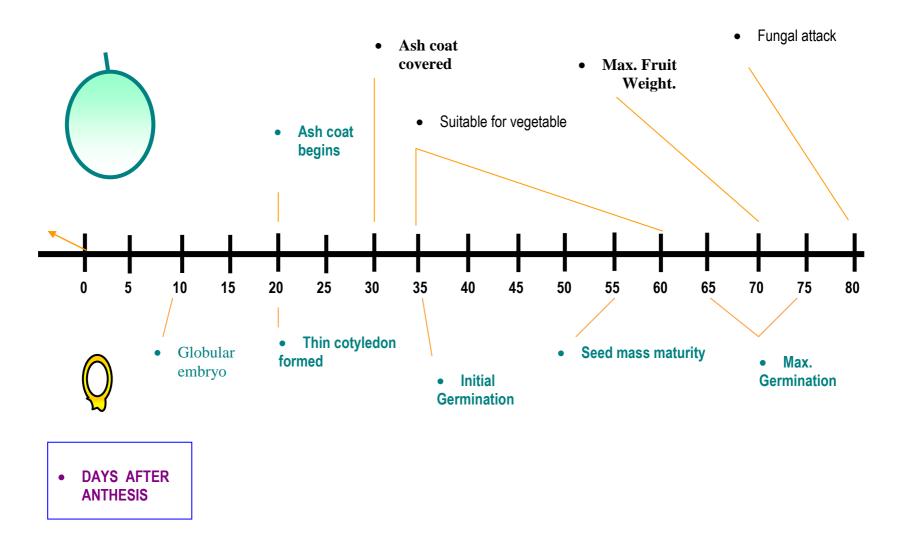
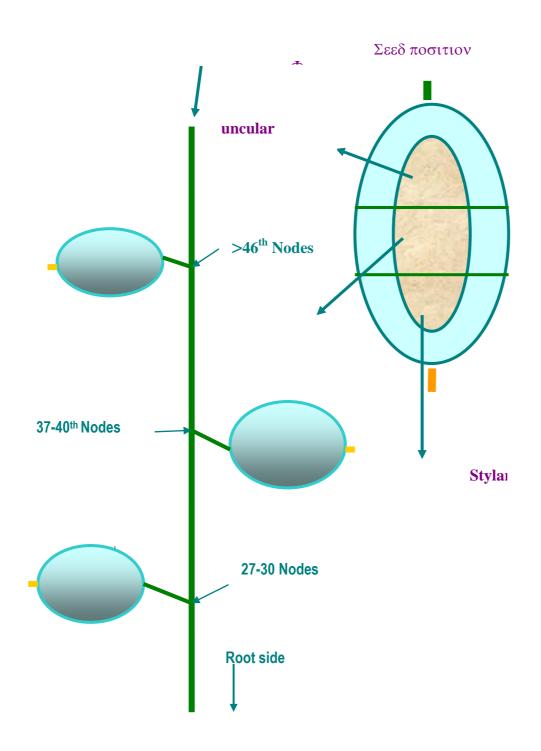


Fig Descriptive comparisons of ash gourd fruit and seed development from anthesis to penultimate fruit and seed maturity during kharif season



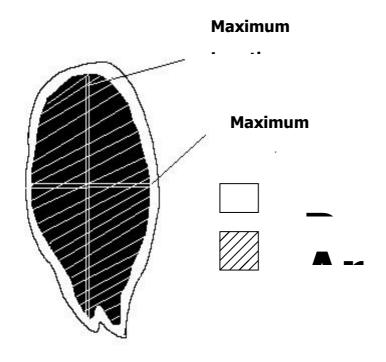


Fig Ash gourd seed top - view with shape descriptors under Image Analysis System

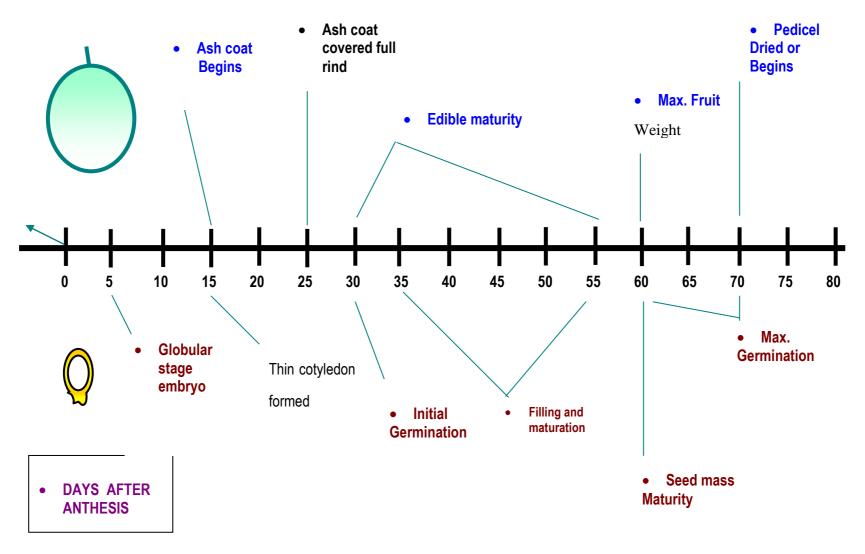


Fig Descriptive comparisons of ash gourd fruit and seed development from anthesis to ultimate fruit and seed maturity during summer season

## ABSTRACT

Harvesting, post harvest handling and conditioning of fruit and seed in ash gourd (*Benincasa hispida* (Thunb.) Cogn)

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Key words: Ash gourd, harvest maturity, post harvest and conditioning

Sowing ash gourd during the month of July (rainy season) was observed best by recording low sex ratio and maximum numbers (five) of fruits per vine. Germination was initiated in ash gourd at 30 days after anthesis, when it was raised in summer season, which was approximately 30 days before mass maturity. In rainy season, seeds from stage 30 Days after anthesis has failed to germinate. Rainy crop maintained the high level of germination (84.5 per cent) upto 80 DAA, whereas in summer it was maintained (76.5 per cent) upto 70 DAA only. Complete crop senescence occurred 170 days after sowing in summer, whereas in rainy season vine and fruit attachment with plants existed beyond 180 days.

Elongated tubular or rod shaped thickening in epidermal cells running in the radial direction from the inside to outside, was visible in the seed coat of matured seeds of ash gourd. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local confirmed their maturity stages, as both cotyledons and seeds of matured stages observed to have more values of seed descriptors studied in Image Analysis System. High molecular weight proteins were totally absent in both the varieties of ash gourd, which could be observed in all the lanes of seed protein bands obtained in electrophoresis

Seeds from peduncular segments of bottom, middle and top fruits borne in the vine produced the lowest germination. Seeds from top fruits outperformed middle and bottom fruits. The mature seeds from top fruits was heavier than those from middle and bottom fruits. Hence, ash gourd fruits from the top nodes (beyond 46<sup>th</sup> node) could be utilised for seed purpose and from the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised for vegetable purpose in rainy season.

Germination of seeds significantly increased from 36.5 and 38.0 per cent (at the time of storage)

to

94.5 and 91.5 per cent after eight months of fruit storage in CO 2 and Kerala local, respectively. After eight to nine months of fruit storage, there was a slight decrease in germination and vigour, indicating the on set of seed deterioration inside the fruit during fruit storage. Fruit storage is recommended for post harvest maturation and to achieve higher germination.

Closed fermentation for 72 h combined with washing for 15 min, drying at room temperature (25°C) for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth and non fermentation gave poor germination and vigour. To

extract one kilogram of seeds,

Rs. 56 was incurred due to closed fermentation method in a large scale seed production unit. The cost benefit ratio was 1:3.1.

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**RESEARCH FINDINGS** 

Harvesting, post harvest handling and conditioning of fruit and seed in ash

gourd (Benincasa hispida (Thunb.) Cogn)

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**Professor and Head** 

Sowing ash gourd during the month of July (rainy season) was observed best by

recording low sex ratio and maximum numbers (five) of fruits per vine. Germination was

initiated in ash gourd at 30 days after anthesis, when it was raised in summer season, which

was approximately 30 days before mass maturity. In rainy season, seeds from stage 30 Days

after anthesis has failed to germinate. Rainy crop maintained the high level of germination

(84.5 per cent) upto 80 DAA, whereas in summer it was maintained (76.5 per cent) upto 70

DAA only. Complete crop senescence occurred 170 days after sowing in summer, whereas in

rainy season vine and fruit attachment with plants existed beyond 180 days.

Elongated tubular or rod shaped thickening in epidermal cells running in the radial

direction from the inside to outside, was visible in the seed coat of matured seeds of ash

gourd. Geometric measurements performed in ash gourd varieties CO 2 and Kerala local

confirmed their maturity stages, as both cotyledons and seeds of matured stages observed to

have more values of seed descriptors studied in Image Analysis System. High molecular

weight proteins were totally absent in both the varieties of ash gourd, which could be

observed in all the lanes of seed protein bands obtained in electrophoresis

Seeds from peduncular segments of bottom, middle and top fruits borne in the vine

produced the lowest germination. Seeds from top fruits outperformed middle and bottom

fruits. The mature seeds from top fruits was heavier than those from middle and bottom fruits.

Hence, ash gourd fruits from the top nodes (beyond 46<sup>th</sup> node) could be utilised for seed

purpose and from the bottom (27-30<sup>th</sup> node) and middle fruits (37-40<sup>th</sup> node) could be utilised

for vegetable purpose in rainy season.

Germination of seeds significantly increased from 36.5 and 38.0 per cent (at the time

of storage) to 94.5 and 91.5 per cent after eight months of fruit storage in CO 2 and Kerala

local, respectively. After eight to nine months of fruit storage, there was a slight decrease in

germination and vigour, indicating the on set of seed deterioration inside the fruit during fruit

storage. Fruit storage is recommended for post harvest maturation and to achieve higher germination.

Closed fermentation for 72 h combined with washing for 15 min, drying at room temperature (25°C) for 72 h and sun drying for 12 h had superior performance, whereas prolonged open fermentation resulted in fungal growth and non fermentation gave poor germination and vigour. To extract one kilogram of seeds, Rs. 56 was incurred due to closed fermentation method in a large scale seed production unit. The cost benefit ratio was 1:3.1.

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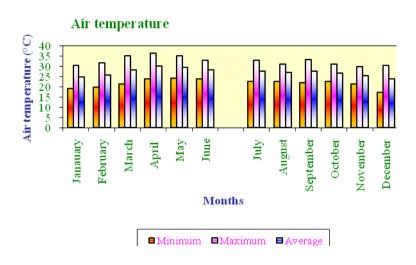
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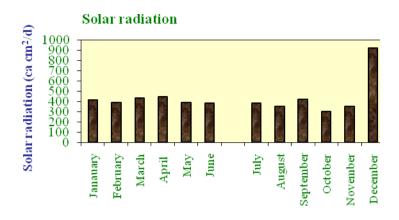
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Figure 3. Weather data recorded during summer and rainy seasons during 2002







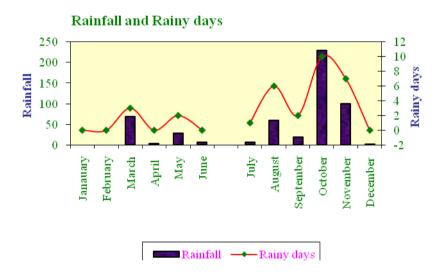
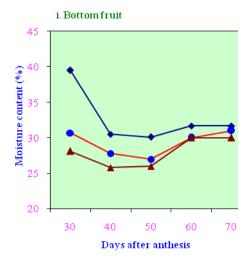
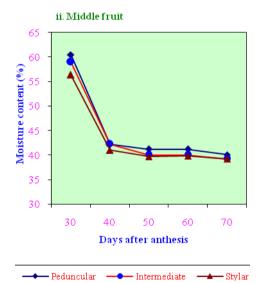
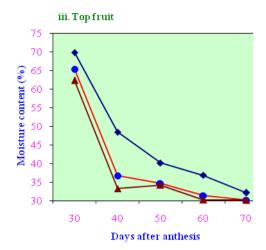


Figure 9. Moisture content and germination of seeds as influenced by fruit and seed position in ash gourd cv.  ${\rm CO}~2$ 

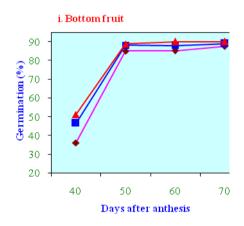
### a. Moisture content

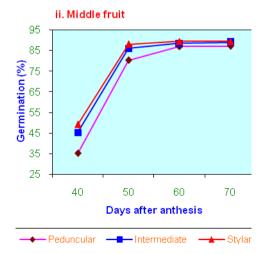






### b. Germination





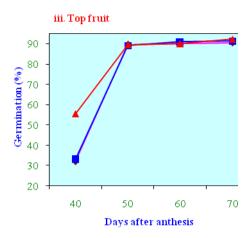
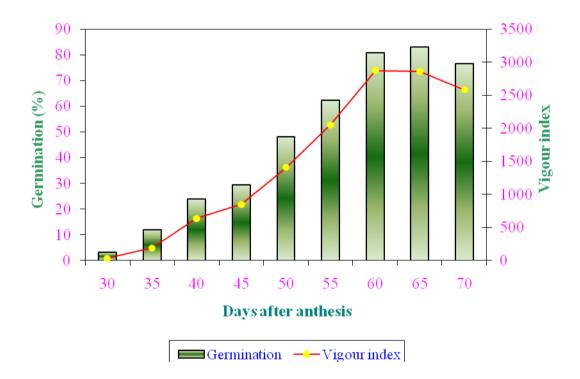


Figure 5. Germination and vigour index at different stages of seed development and maturation in ash gourd



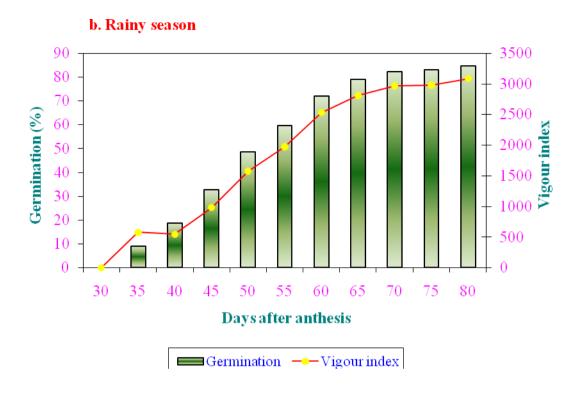
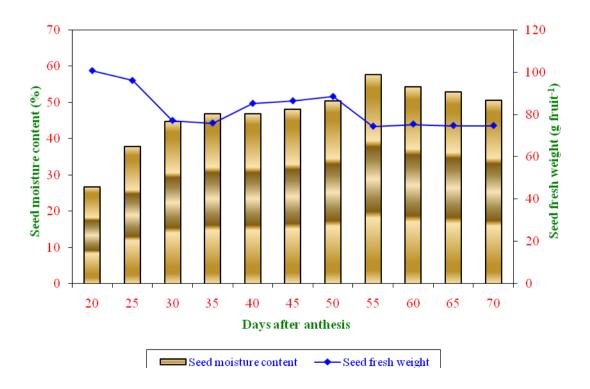


Figure 4. Seed moisture content and seed fresh weight at different stages of seed development and maturation in ash gourd



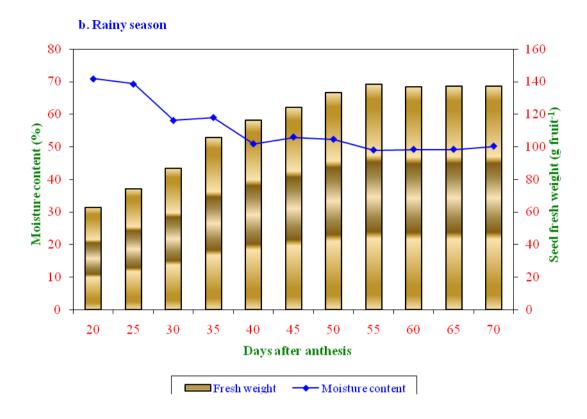
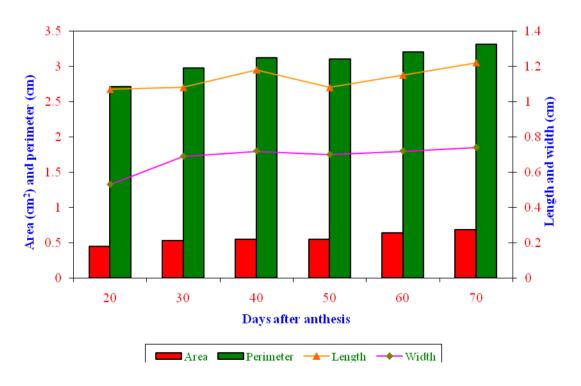
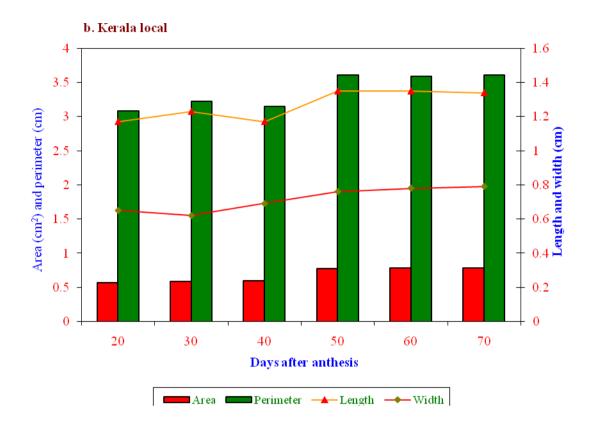
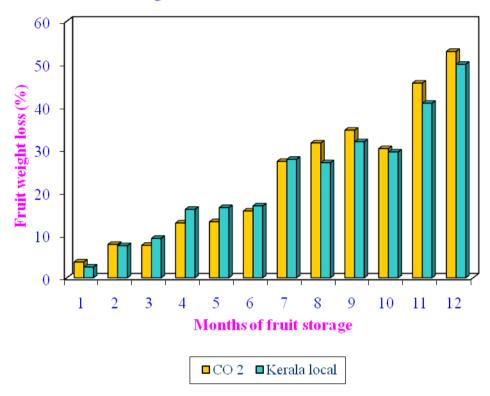


Figure 8. Area, perimeter, length and width of seed measured in immage analysis system at different stages of development and maturity in ash gourd varieties





## a. Fruit weight loss



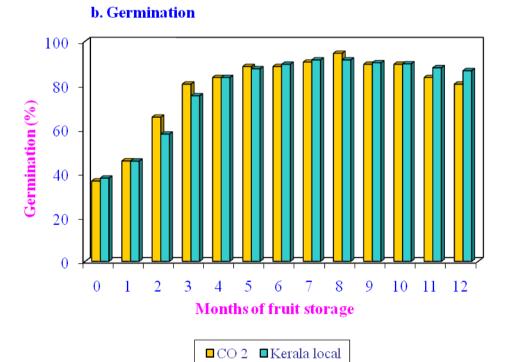
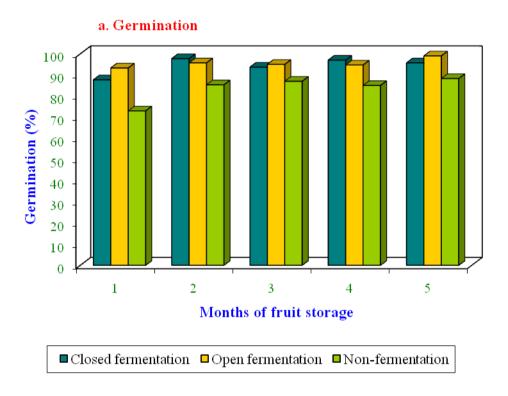
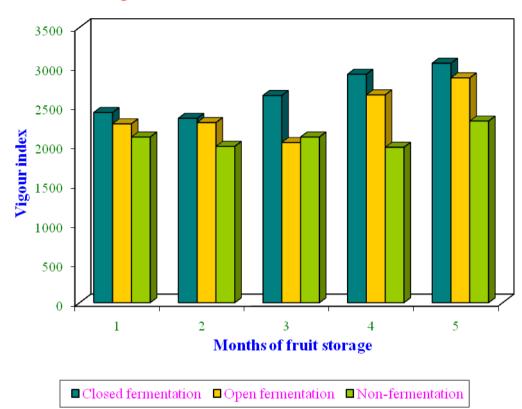


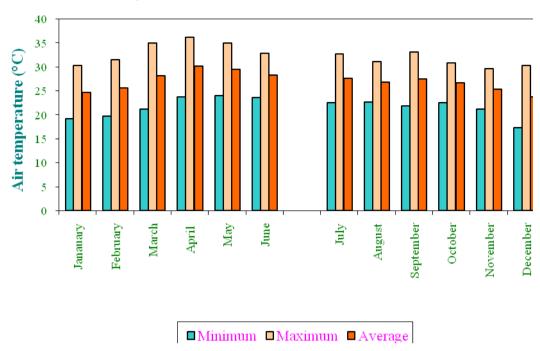
Figure 11. Germination and vigour index in closed fermentation, open fermentation and non-fermentation combined with different washing and drying treatments in ash gourd cv. CO 2 during rainy season



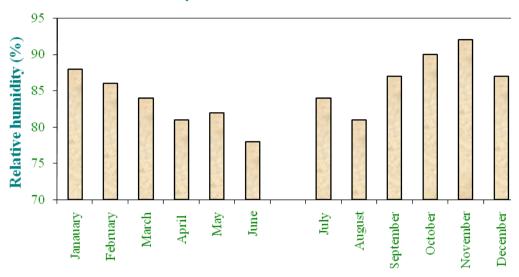
# b. Vigour index



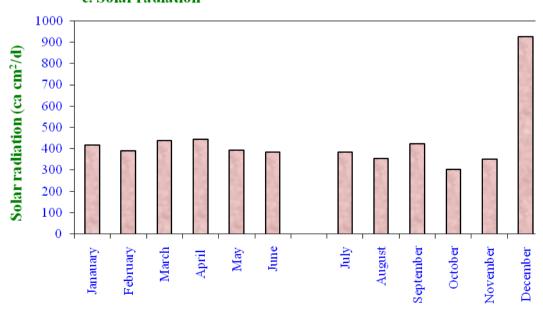
# a. Air temperature



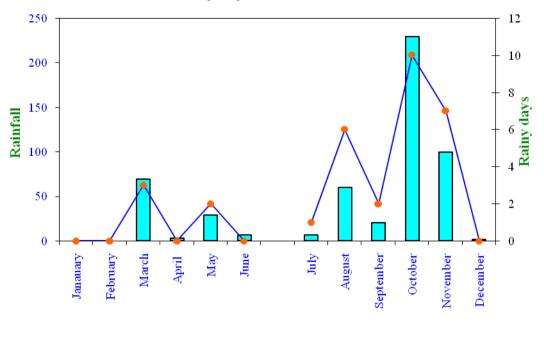
# b. Relative humidity



## c. Solar radiation



# d. Rainfall and rainy days



■Rainfall ——Rainy days

Plate 12 a. Structural changes in the embryo at different stages of development and maturation in ash gourd cv CO 2 during rainy season

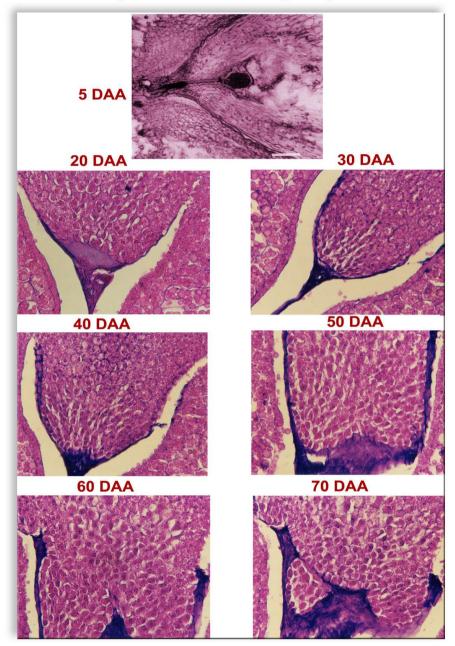


Plate 12 b. Structural changes in radicle during seed development and maturation in ash gourd cv. CO 2 during rainy season

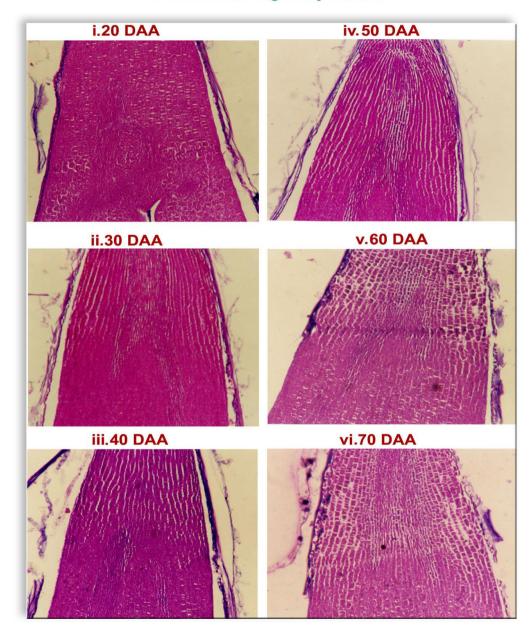


Plate 11 : Structural changes in seed coat at different stages of development and maturation in ash gourd cv.

CO 2 during rainy season

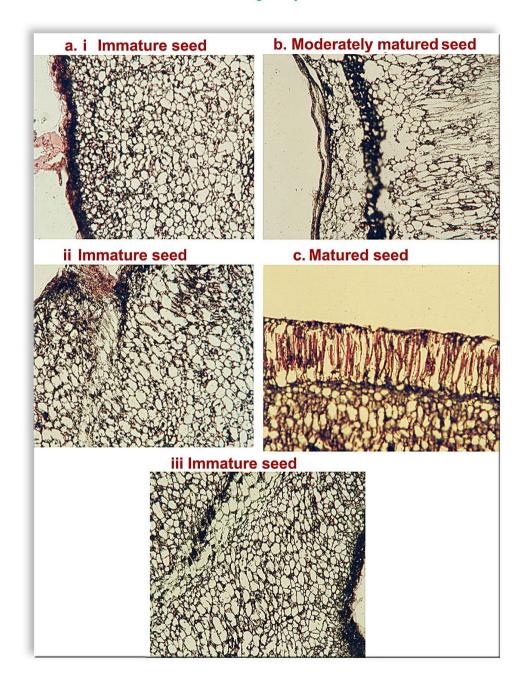
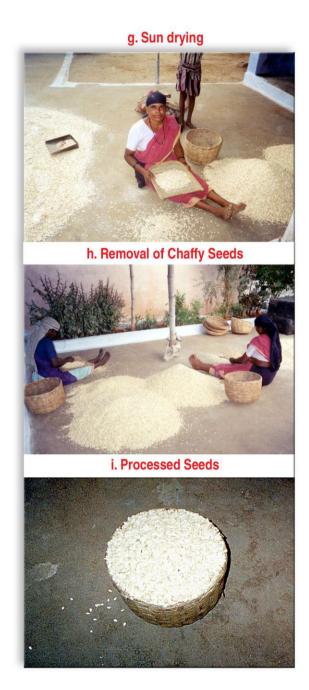


Plate 16: Step by step process executed for closed fermentation, washing and drying during On Farm Trial at Kondamuthunur of Erode district











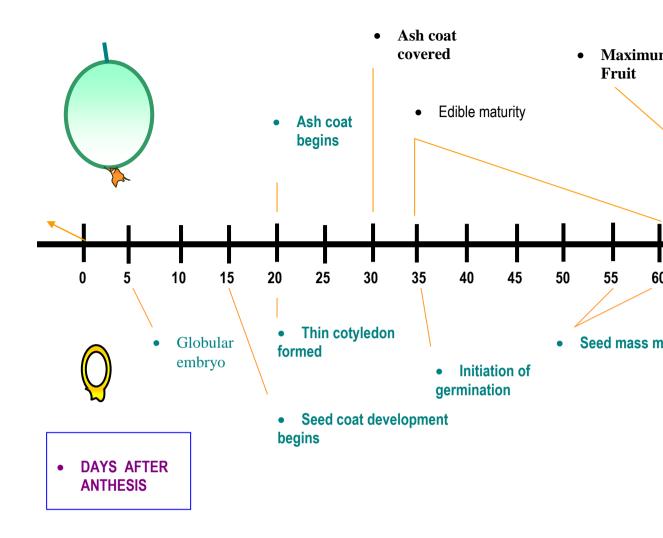


Fig.7 Comparison of descriptive characteristics of ash gourd fruit and seed during development season

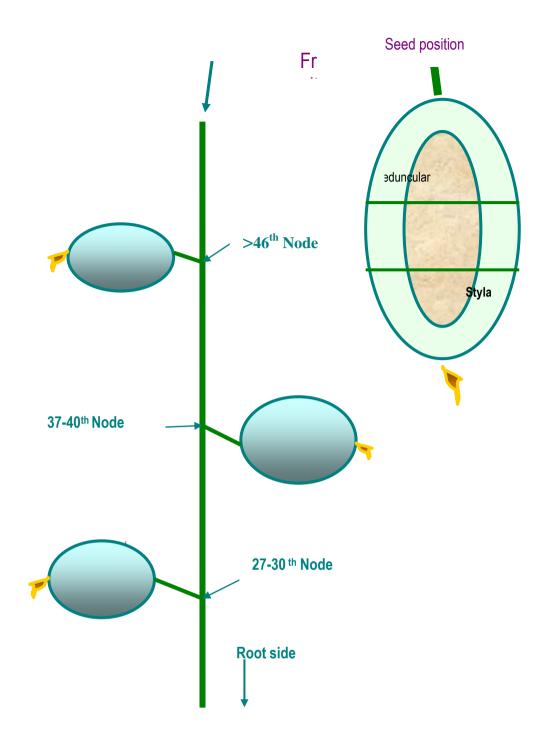


Fig.2 Schematic diagram of ash gourd vine representing three fruit position viz., top, middle and bottom and three seed position viz., peduncular, middle and stylar in the seed cavity

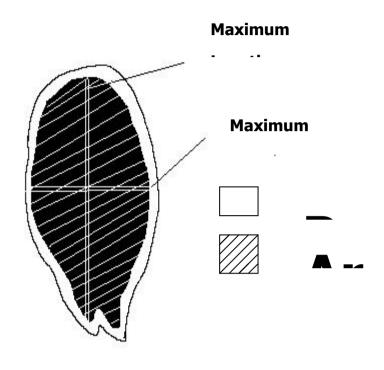


Fig.1 Ash gourd seed top - view with shape descriptors under Image Analysis System

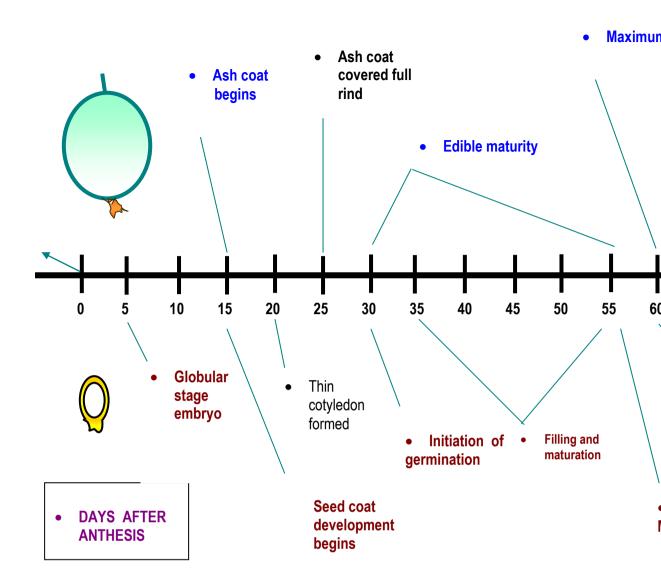


Fig. 6 Comparison of descriptive characteristics of as