





Advances in Summer Squash (*Cucurbita pepo* L.) Molecular Breeding Strategies

8

Amira A. Ibrahim , Khaled F. M. Salem , Mohamed A. Abdein, and Samah M. Ramadan

Abstract

Summer squash (*Cucurbita pepo* L.) is a self-pollinated crop belonging to Cucurbitaceae. It is an annual crop that is grown up in tropical and subtropical areas. It is one of the most vital and economical vegetables in cultivation. Globally, squash is used as food and medicine for the presence of vitamins and antioxidants. *C. pepo* plants vary in shape, color and size, with several varieties and landraces. There are many types of *Cucurbita* species with diverse genomes and chromosome numbers. The number of summer squash chromosomes (2n) ranges from 40 to 48. Squash harbors a great diversity dependent on ploidy, regional and morphological characteristics. The introduction of new alleles

A. A. Ibrahim

Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, Alexandria, Egypt
e-mail: aabdallh@srtacity.sci.eg

K. F. M. Salem (✉)

Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Egypt

Department of Biology, College of Science and Humanitarian Studies, Shaqra University, Qwaieah, Saudi Arabia

e-mail: khaledfathi@su.edu.sa; khaled.salem@gebri.usc.edu.eg

M. A. Abdein

Biology Department, Faculty of Arts and Science, Northern Border University, Rafha, Saudi Arabia

Vegetables Breeding Department, Horticulture Research Institute, (HRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Giza, Egypt

e-mail: mohamed.abdein@nbu.edu.sa

S. M. Ramadan

Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

through the crossing of different genetic resources for the *C. pepo*, for example, crossing common genotypes with locally developed genotypes with those developed locally increases genetic diversity and the preselection of characteristics of interest. A fair natural variability in the degree of phenotypes must be ensured. The main targets of squash breeders and geneticists are to enhance various desirable morphological characteristics, including tolerance to biotic and abiotic stress and crop characteristics. Achieving these targets can be comforted by using modern genomics methods to enhance the traditional breeding program. This chapter provides an outline of the shortcomings of the summer squash origin and historical background, botanical description, economic and health importance, photochemistry, cultivation requirements, biodiversity and conservation of germ-plasm, cytogenetics, aims and stages of squash breeding programs and traditional approaches of breeding. Additionally, it discusses new plant breeding methods involving marker-assisted breeding and genetic engineering.

Keywords

Biodiversity · Biotechnology · *Cucurbita pepo* · Genetic improvement · Genetic map · Modern breeding · Summer squash (*Cucurbita pepo* L.) · Traditional breeding

8.1 Introduction

Summer squash (*Cucurbita pepo* L.) is an essential component of a balanced and safe diet. It contains large quantities of vitamins, antioxidants and other ingredients which avoid disease and promote healthier living standards. Summer squash is the edible green fruit of the Cucurbitaceae family of gourds, a very diverse genus of *C. pepo*. It becomes ready for harvest when it is shiny. Typically, the recommended size varies from 100 to 200 grams. This size is often reached between 2–5 days after anthesis depending on growing conditions. They keep growing and start losing their shininess unless the fruits are harvested on time. Usually, oversized fruit is unsaleable. Summer squash is a short-season crop, simple to grow and ideal for tropical and subtropical areas, with some species extending to the temperate zone (Ittah and Kwon-Ndung 2019; Ndoro et al. 2007; Subrahmanyam 2004). According to the last taxonomical treatment, cucurbits belong to Cucurbitaceae and contain about 118 genera and 825 species (Jeffrey 1990). Cucurbits are found in both the new and old worlds and are amongst the essential plant families that produce edible foods and beneficial fibers to humans. Cucurbits are composed of five subfamilies, i.e., *Fevilleae*, *Melothrieae*, *Cucurbitaceae*, *Sicyoideae* and *Cyclanthereae*. *Cucurbita*, *Cucumis*, *Citrullus*, *Lagenaria* and *Luffa* were the most important cultivated genera in the subfamily Cucurbitaceae and *Sechium* of the Sicyoideae subfamily (Whitaker and Davis 1962).

The summer squash is *C. pepo* eatable undeveloped fruit that contains various species belonging to the Cucurbitaceae family. Squash is one of the easy and simple

crops to plant, has the yield of a short season and can grow in tropical and subtropical regions (Paris 1996). The *C. pepo* is considered one of the Middle East's most well-known vegetable crops and its common name is Kosa, well-known among Egyptians, who coined this (Abdein 2016). Summer squash belonging to the species *C. pepo* contains numerous varieties. These varieties show variations in their vegetative features, flowering times, yield and components traits. The fruits are also highly edible in length, diameter, form and color. Thus, squash breeders will find a great variation among all summer squash germplasm collections (Abdein 2016; Moon et al. 2019).

8.2 Origin and Distribution

Bailey (1929) coined a cucurbit term for cultivated species within the Cucurbitaceae family. In the Arabic language, the word Qaraayat is used to identify a Cucurbitaceae cultivated species. Many species of Cucurbitaceae have been domesticated in pre-historic times (Kochhar 1981). Cucurbits have been cultivated for centuries. Several recent cultivated types are unknown in the wild and their origin has been difficult to trace (Herklots 1972).

The cucumber is of Asian descent, first found in Nepal's Himalayan foothills. Cucumber remains dated from the third millennium BC in Eastern Iran. Early travelers took cucumber to Mediterranean countries 3000–4000 years ago, where the fruits were revered by the ancient Romans and Egyptians. Since the ninth century, the cucumber was known in France and was cultivated in the early days of the fourteenth century in Britain. Columbus introduced it to the New World, planting it in Haiti in 1494 and it was presumably transported to the USA afterward (Decker-Walters et al. 1999; Thompson and Kelly 1957; Wehner and Robinson 1991; Whitaker and Davis 1962).

Sinnott and Durham (1922) made the first attempt at a thorough genetic study of the cucurbits. Many botanists claim that two *Cucurbita* species, vegetable marrow *C. pepo* and squash *C. moschata* Duch are of American origin and pumpkin *C. maxima* are of Asian origin (Thompson and Kelly 1957). Archaeological evidence positions wild populations of *C. pepo* about 10,000 and 30,000 years ago in Mexico and the eastern USA, respectively (Decker 1988).

Summer squash is belonging to the *Cucurbita* genus and the Cucurbitaceae family (<https://www.itis.gov>). *Cucurbita pepo* L. subsp. ovifera (L.) D. S. Decker var. ovifera (L.) Harz; *Cucurbita pepo* L. subsp. pepo var. pepo; *Cucurbita pepo* L. var. montia Duch; *Cucurbita pepo* L. var. patisson Duch; *Cucurbita pepo* var. giraumontia Filov; *Cucurbita pepo* var. melopepo (L.) Harz; *Cucurbita pepo* var. styriaca Greb are all synonyms for *Cucurbita pepo*. Summer squash, vegetable marrow, field pumpkin, jack-o-lantern pumpkin, zucchini, cocozelle, citrouille, (*Cucurbita pepo* L.) is a flowering plant within the class Magnoliopsida, order Cucurbitales and family Cucurbitaceae (gourds, squashes, citrouilles, gourdes). Summer squash (*Cucurbita pepo* L.), vegetable marrow, field pumpkin, jack-o-lantern pumpkin, zucchini, cocozelle, citrouille is of the *Cucurbita*.

Kingdom: Plantae —Plantes, Planta, Vegetal, plants
Subkingdom: Viridiplantae —green plants
Superdivision: Embryophyta
Division: Tracheophyta —vascular plants, tracheophytes
Spermatophytina —spermatophytes, seed plants, phanérogames
Class: Magnoliopsida —Dicotyledons
Superorder: Rosanae
Order: Cucurbitales
Family: Cucurbitaceae —gourds, squashes, citrouilles, gourdes
Genus: Cucurbita L.—gourd
Species: Cucurbita pepo L.—vegetable marrow, field pumpkin, jack-o-lantern pumpkin, zucchini, cocozelle, citrouille
Subspecies <i>Cucurbita pepo</i> ssp. <i>pepo</i> L.
Summer squash

8.3 Botanical and Distribution

Cucurbits are annual herbaceous or perennials that take care of the roots and usually the wet vines. The plants grow either level along the ground or use rings to ascend. They rarely grow as trees, shrubs or hedges. The curls may render fanned or guided, produced at the base of the petiole. Four twisted strands were usually curling with a concrete surface. Take-off has changed from essential to a compound of palmate. Those get out that, grow a while later, are lobed even more comprehensively and extra-natural nectars are found now and again. The sprouts are unisexual, and the plants are male or female, to be hermaphroditic occasionally.

Numerous perpetual cultivars have tuberous roots or pachypodia and in an annual period, their herbaceous shoots kick the bucket and re-develop (Hemicryptophyte life frame). Pachypodia can reach the broadness of one meter (Olson 2003). There are also several shrub species within the family (*Acanthosicyos horridus* Welw. ex Hook. f., *Corallocarpus glomeruliflorus* (Deflers) Cogn. *Momordica Spinoza* (Gilg) Chiov and lianas up to 10 cm (Carlquist 1992) with woody and perennial stems. Neel et al. (2017) point out that *C. pepo* is a creep or climbing annual plant with five diagonal stems up to 15 m long. Branches outside the shallow root chain, grow from well-developed roots. The stems are rough and hairy at the nodes, branching between 6 to 24 cm long, and usually rooted. The plant has tendrils at 90° when inserting the rolled and branched leaf 1–6. Tendrils can be formed poorly on dense plants. The leaves are flat, alternating, wide oval to deltoid, generally cardioid, apical pointed, palm ally lobulated with 5–7 lobes, slightly dented, rough, palm-veined, 20–30 cm length and 10–35 cm wide leaves with 5–25 cm small leaves which are oval elliptical heart to suborbicular cordate, with or without white spots on the surface and have 3 to 5 rounded or obtuse lobes, apical lobes, middle lobes. Petioles that cordately support sub-orbicular-cordate, superficially with or without white spots and have 3–5 modified or unnatural, apiculate lobules, the focal lobules invited



Fig. 8.1 Differences of some new, fresh squash leaves. (Source: Photo by M. A. Abdein)

than level ones. Squashes are monoecious and have single, nectar-giving actinomorphic blooms. This species of flowers usually have three stamens. The female flowers have thick peduncles, 3 to 5 cm long. It is also elliptical to the curved, multilocular ovary, sepals which are rarely fallacious and have a larger corolla than the male, with flowers having three lobate stigmas (Fig. 8.1). Cucurbit fruits are distinct in shape, color and measure (Yadav et al. 2017). On 1 or 2 closes it may be oval, round and hollow, scalloped, globular, fusiform, straight or possibly decreasing to a twisted or straight neck. Also, fruits have different colors (white, yellow, light to dark green, nearly black, creamy and/or orange) with smooth or rough skin. The pulp also varies in color (white, yellow or orange) and thickness (Figs. 8.2 and 8.3). Different *C. pepo* characteristics are shown in Table 8.1.

8.4 Economic Importance, Uses and Health Benefits

As a vegetable crop squash has valuable economic importance. World pumpkin, squash and gourd production quantities are 27.45 million tons over a total area of approximately 20.78 million hectares. Pumpkins, squash, and gourds are produced worldwide commercially in many countries and are cultivated in most places as a vegetable (FAO 2018) (Fig. 8.4a). It is especially important in Asia, Europe, America and Africa as a commercial crop (FAO 2018) (Fig. 8.4b). China, India, Russia, Ukraine, USA, Egypt, Mexico, Malawi, Italy and Spain are the top ten pumpkins, squash and gourds producers worldwide in 2018 (FAO 2018) (Fig. 8.4c). The pumpkin, squash and gourd production are shown in (Table 8.2).



Fig. 8.2 Male and female flowers of *Cucurbita pepo*. (Source: Abdein 2016)

The Cucurbitaceae family includes pharmaceutically important plants. Cucurbits are a group of sound nourishments. Cucumbers are used as nourishment, significantly consuming fewer calories. It has 96% water, a little sugar and multiple calories figuratively speaking. By extension, it provides an incredible wellspring of nutrients A, K and C as well as full potassium. National cancer coordinated considered cucumbers as one of the pharmaceutical properties to have disease defense advantages. Cucurbits are the most important basic plant families which offer appropriate things and important filaments to individuals. Cucumbers are either rough or salted to exhaustion. Pickling may be a standard approach for sparing the cucumber for long times. It allows them to be available long after the creating regular season. Cucumbers are always soaking in brine, vinegar or some other flavor. The cucumber jam was obtained, just as it was instilled with special flavors (Perez Gutierrez 2016). This family has huge medicinal and pharmaceutical properties that are hostile to HIV, anxiolytic, pyretic, diarrhea-hostile, carminative, cancer-preventive, diabetic, antibacterial, diuretic, anthelmintic, tuberculosis-hostile and laxative. It is used as an abortive, diuretic and cardiotoxic head to boot. They do tend to have strong properties soothing, antitussive, cytotoxic and expectorant (Saboo et al. 2013). Cucurbitaceae includes vegetables such as squashes, melons, cucumbers and luffas. Cucurbits shape a vitally important and huge group of vegetables produced in the subtropical and tropical nations. This family has many pharmaceutical and nutritional plants (Gill and Bali 2012). In standard and alloxan-diabetic rats (Kolawole et al. 2011), natural products of cucumber flowers function

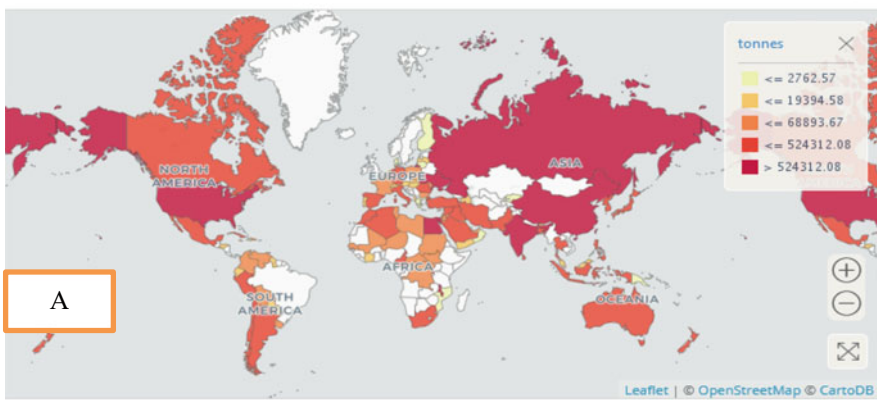


Fig. 8.3 Various fresh fruits color and shape of *Cucurbita pepo*. (a) Various fresh fruits and (b) Different shapes of ripening fruits. (Source: Abdein 2016)

Table 8.1 Different summer squash *Cucurbita pepo* characteristics

Traits	Degree of traits
Cotyledon leaves shape	Oval, Wide circular rectangular, Circular rectangular narrow
Nature of plant growth	Short, Medium Stretch, Dispenser
Discharge	Exists, Does not exist
Degree of Discharge	Poor, Medium, Strong
Direction of petiole	Erect, Semi erect, Horizontal
Stem length cm	Short (>50), Medium (50–100), Long (<100)
The green color pigment of the vein	Light green, Medium, Dark green
The presence of the veins	Absent, Archaeological, Developing well
Number of plant leaves	Little (15–25), Average (25–35), Many (>35)
Leaves pin nation	Absent, Weak, Medium, Strong, Very strong
The green color pigment of the upper layer of leaves	Light green, Medium, Dark green
Leaf area (cm ²)	Small (>500), Medium (500–1000), Large (>1000)
Greenness of the petioles	Light green, Medium, Dark green
Length of petioles (cm)	Short (<30), Medium (30–50), Long (>50)
The thickness of petioles (mm)	Thin (<30), Medium (30–40), Thick (>50)
Cross-section of petiole	Polygon, circular
The presence of thorns (spines) on the petiole	Little, Average, Many
Length of masculine syphilis (cm)	Short (<25), Medium (25–30), Long (>30)
Color of the male flower	Yellow, Orange
Color of the female flower	Yellow, Orange
Fruit length cm (age combined)	Short (<10), Medium (10–15), Long (>15)
Fruit diameter cm (age combined)	Thin (>3), Medium (3–5), Wide (>5)
Fruit shape index (fruit length/fruit diameter)	Small (<0.5), Medium (0.5–1), Large (>1)
The presence of a neck with fruit	Absent, Exist
Young fruit color	White, Yellow, Creamy, Dark green, Light green; Green-striped
The shape of the cross-section of the young fruit	Lobular, Ribbed, Circular
The shape of the fruit	Cylindrical, Rectangular, Synthetic, Disc, Thumb, Spherical, Cardiac, Twisted Neck
Color of mature fruit	White, Yellow, Creamy, Dark green, Light green, Green, striped
Color density in mature fruit	Light, Medium—Bold
The length of the fruit is mature (cm)	Very short (<15), Short (15–20), Medium (20–25), Long (25–30), Very long (>30)
Fruit diameter cm (ripe fruit)	Small (<10), Medium (10–15), Wide (>15)
Fruit mature shape index (the length of mature fruit/diameter of mature fruit)	Small (<0.5), Medium (0.5–1), Large (>1)
Seed shape	Rectangular Rectangle, Rectangular, Rectangular Rectangle
Seed color	White, Yellow

Prepared by M. A. Abdein



Production share of Pumpkins, squash and gourds by region

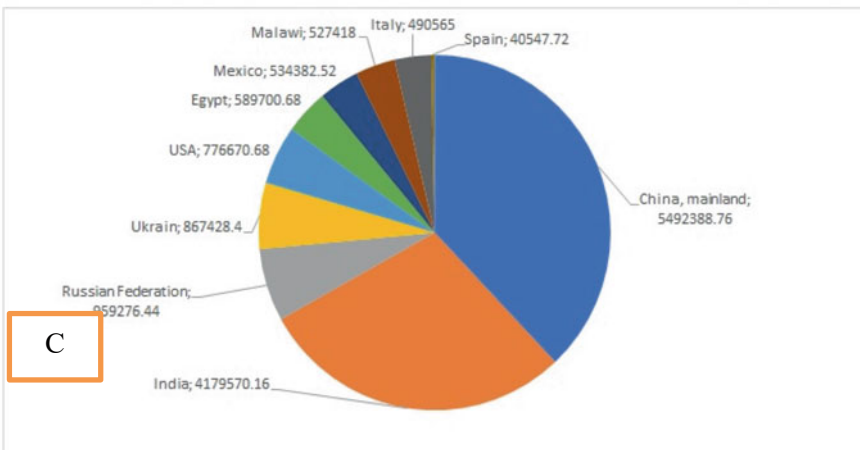
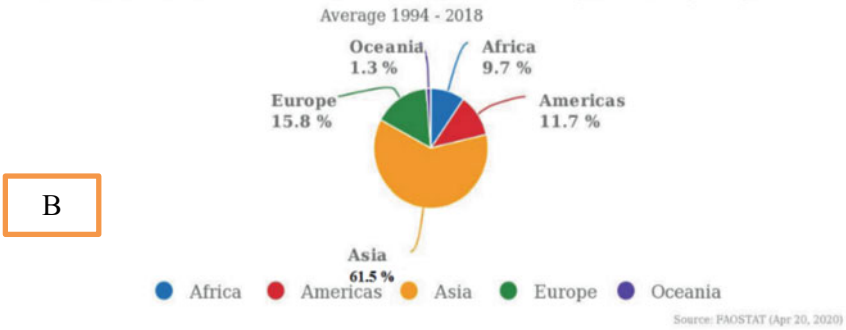


Fig. 8.4 Pumpkins, squash and gourds worldwide production. (a) Top ten producers of pumpkins, squash and gourds worldwide, (b) Map of World pumpkins, squash and gourds production and (c) Important region of pumpkins, squash and gourds. (Source: FAO 2018)

Table 8.2 World pumpkins, squash and area harvested, production and yield in 2017

Location	Area harvested (ha)	Production (tons)	Yield (hg/ha)
World	2,078,450	27,449,481	132,067
Americas	210,115	3,427,339	163,118
Asia	1,332,071	16,623,732	124,796
Europe	177,266	4,213,743	237,708
Africa	344,755	2,938,604	85,238
Australia and New Zealand	11,785	190,417	224,403

Source: FAO, FAOSTAT agricultural database. <http://apps.fao.org> 2017

as an antidiabetic specialist and can be utilized as an antiviral treatment (Puri et al. 2009). In addition, it has antimicrobial, cell reinforcement (Leelaprakash et al. 2011) and anti-tumor activities on human nasopharyngeal carcinoma cells in vitro and in vivo (Fang et al. 2011). Cucurbit plants have been successfully used to treat different diseases as traditional, homegrown cures. They also demonstrated exercises that are anti-inflammatory, anti-tumor, hepatoprotective, cardiac and immunoregulatory. In common, these family individuals are well off the protein source, with various natural works being performed as antibacterial, antiviral, antifungal, antitumor, antidiabetic and anti-aids. Various bioactive compounds are found in cucurbits such as triterpenes, cucurbitacin, sterols and alkaloids (Ajuru and Nmom 2017).

Cucurbita pepo is used in many nations as an anti-inflammatory, antiviral, analgesic, antiulcer, antidiabetic and antioxidant, for example, to treat a few diseases. *C. pepo* is known for its natural product and seeds used for consumption. It has white seeds embedded within the shell. These seeds are chewable and have a slightly sweet taste. The benefit of handling squash seed comes from its stimulating effect on dislocation of the bladder and sphincter. Its seeds have been shown to infringe mononuclear blood cells in vitro by the immunosuppressive movement (Winkler et al. 2005). Furthermore, as antibacterial agents (Obi et al. 2009). Gill and Bali (2012) reported that seeds of *C. pepo* have antiulcer and antioxidant activities due to the tetracyclic and triterpenoids (cucurbitacin) material. Uses of different parts of cucurbits were illustrated in Table 8.3 and Fig. 8.5.

8.5 Photochemistry

Squash is one of the most critical health sources as it includes many diverse bioactive elements, for example, polysaccharides, corrosive para-aminobenzoic, settled oils, sterols, proteins and peptides (Caili et al. 2006; Murkovic et al. 2002; Salehi et al. 2019). *C. pepo* seeds have squalene concentrations of 583.2–747 mg/100 g. Squalene can be a triterpene produced by humans, plants and animals. In their biosynthesis inside the human body, it could be a predecessor of steroid hormones, cholesterol and vitamin D. Squalene also has significant effects on various cancer treatment

Table 8.3 Squash parts and common uses

Parts	Traditional uses	References
Fruit	Cooling and astringent to the bowels, cure fatigue and thirst	Acosta-Patino et al. (2001)
	Increases appetite, cures leprosy and purifies the blood	Saboo et al. (2013)
Seed	Cure sore chests, hemoptysis, bronchitis and fever	Winkler et al. (2005)
	Biochemical immune mechanisms are caused by interferons, hyperplasia of the prostate and antioxidant capacity	Abdel-Rahman (2006)
	Oil from squash seeds has long been used to alleviate challenges related to an expanded prostate organ and touchy bladder-related micturition issues	Williams et al. (2006) Obi et al. (2009)
	Treatment decreases the side effects of micturition but does not decrease the increased prostate organ volume. It has antioxidant movement (inhibitory movement against lipid peroxidation and free radical rummaging), the androgenic activity of the insects, immunological movement, antiviral movement, cardiovascular movement, anti-inflammatory activity, and hepatoprotective motion	Gill and Bali (2012)
	Often, it triggers inhibition of prostate hyperplasia caused by testosterone	Müller and Bracher (2015)
Leave	Reduced fever, nausea medication and a boost to blood hemoglobin content	Ratnam (2017)

Prepared by A. A. Ibrahim

**Fig. 8.5** Fresh *Cucurbita pepo* seeds from mature fruit. (Source: Photo by M. A. Abdein)

types (Martha and Gutierrez 2016). The chemical components of the different parts of the squash are shown in (Table 8.4).

Table 8.4 Plant part and chemical composition in squash

Plant part	Chemical composition	References
Fruit	Low fat content (2.3%), carbohydrate content (66%), protein content (3%), high carotenoid content 171.9 µg/g	Elinge et al. (2012)
Seed	Natural source of phytosterols, proteins, polyunsaturated fatty acids, antioxidant vitamins, tocopherols, carotenoids and other elements	Phillips et al. (2005)
	Fatty corrosive components such as palmitic (10.68%), palmitoleic (0.58%), stearic (8.67%) oleic (38.42%) linoleic (39.84%), linolenic (0.68%), gadoleic (1.14%), add up to immersed greasy acids (19.35%) and add up to unsaturated greasy acids (80.65%). Approximately 50% corrosive oil (generally linoleic and oleic), with the most active compounds being Δ7 sterols (avenasterol, spinasterol) and Δ5 sterols (sitosterol, stigmasterol). Triterpenoids, sesquiterpenoids, squalene, and tocopherols (α tocopherol) are also added. Elements include phosphorus, potassium, magnesium, calcium, iron, zinc and trace elements)	Glew et al. (2006); Sabudak (2007)
	Fiber, protein, β-carotene, carbohydrates, minerals and greasy acids were reported in the coat, tissue, seeds and fatty seeds, and triglyceride greasy corrosive blend, tetrahydrothiophene, linoleic corrosive, cholesterol and 13(18)-Oleanen-3-ol. Vitamins (thiamine, riboflavin, niacin, pyridoxine and pantothenic acid), phenolic glycosides and lignan	Stevenson et al. (2007); Ukiya et al. (2002)
Leave	Secondary bioactive compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, tannins, saponins, steroids, gums, mucilage, fixed oils and fats, proteins and amino acids	Kalaiselvi and Selvi (2016)

Prepared by D. M. Hikal

8.6 Nutritional and Genetic Studies

Squash fruit and its peels contain moderate amounts of mineral salts as nutritional compounds used to feed humans. Because of its high content of fibers and vitamins, it can be eaten or cooked as an immature fruit or consumed as a good source of fats and proteins from mature seed.

In a new study, Hikal and Abdein (2018) used the dried rinds of some squash genotypes as antioxidants when applied to the treated cake (Fig. 8.6). It can be eaten as an immature fruit due to its high amount of fibers and vitamin content or used from fully grown seeds as a good resource of fats and proteins. They used four Egyptian and exotic squash varieties Escandarani (Egyptian), Siyah Kabuk (Turkey), Erbil Garden (Iraq) and Zucchini Alberallo Di Sarzana (Italy). The sugar content from the cake and organoleptic properties have been reported under the effect of various peel additives. According to 4 × 4 half mating crosses, different

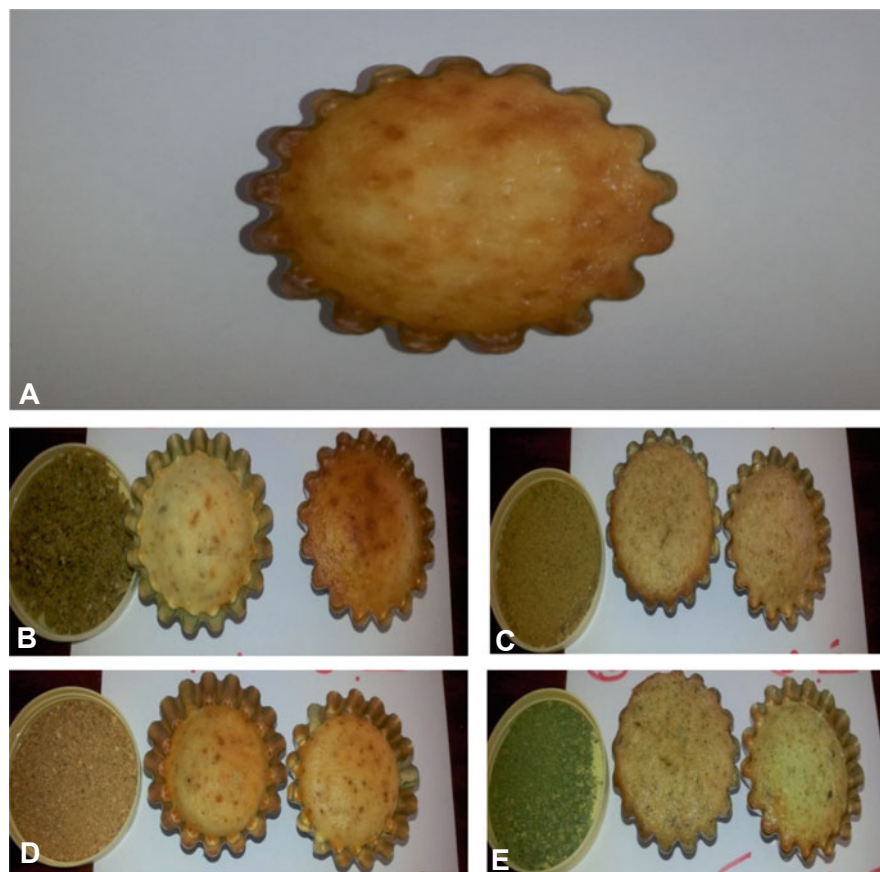


Fig. 8.6 Samples of cake treated with squash peels for several genotypes. (a) Control cake, (b) Cake treated with the squash parent 3 peels (10/250 g and 5/250 g) from left to right, (c) Cake treated with squash parent 4 peels (10/250 g and 5/250 g) from left to right, (d) cake treated with squash parent 2 peels (10/250 g and 5/250 g) from left to right and (e) cake treated with squash parent 1 peel (10/250 g and 5/250 g) from left to right. (Source: Hikal and Abdein 2018)

cultivars were also coated to obtain 6 F_1 crosses. The results showed that different pumpkin skins improved their acceptability and sugar content. On the other hand, the amounts of hybrid activity showed the great importance of all the traits studied compared to the intermediate parents. Estimates of the strength of the hybrid versus the best kinship were very important.

8.7 Cultivation and Conventional Breeding

8.7.1 Recent Cultivation Procedures of the Summer Squash

Depending on the relations between the genotype (the distinctive features of the crop), the environment (climate and soil conditions), management, crop growth and yield can be improved. In warm weather, cucurbits had to be developed to increase the best production. It can also grow in different types of soil, in well-fertilized soil, giving maximum yield. Suitable soil pH ranged from 6 to 6.5. It needs well irrigation for seed germination, with a continuous water supply (Napier 2009). For the preminent reasonable seed germination, the soil cannot be colder than 16 °C and must be between 18.3 °C and 24 °C. Soil temperatures from 18.3 °C to 29.4 °C, are idealized for the most exceptional growth of the plants. Squash is one of the most sensitive vegetables to rainfall and rainfall deficiency and over-irrigation (OECD 2016).

8.7.2 Current Agricultural Challenges

Squash is manually harvested. It is regularly planted in the soil in glasshouse conditions utilizing a drip irrigation technique in the spring, summer, and fall. The physical properties are fundamental in optimizing the productivity of squash seeds for different variables, sifting skills, and moving pneumatics. The bulk thickness of squash seed and part of the various dampness ranges changed individually from 350–475 to 406–460 kg/m³. Simultaneously, the porosity increased from 22.2 to 24.0% as the dampness grew significantly from 6.46 to 52.9%. The burst quality of the dampness material was excessively subordinate (Rouphael and Colla 2009).

Generally, seeds had to be fragile at high humidity content and consequently, cracking required less restriction. Higher temperatures and sun-powered radiation from spring to summer and less concentrated fertilizer arrangements should be used to keep the average electrical conductivity (EC) increasing to the required degree to avoid a delivery drop. In expansion, our consideration has appeared that in the summer to fall season both water system frameworks can be adopted using supplement arrangement, composition 1.8 deci-Siemens per meter (dS/m), but under certain water systems, higher natural product quality (dry matter and carbohydrates) and a decrease in component concentration in the arrangement in the course of the growth process leading to the rearrangement of the closed circle supplement solution administration (Bahlgerdi 2014).

To induce the most noteworthy natural product to abdicate in the summer squash established in field conditions, the impacts of irrigation water quantity, plant water utilization and interim water system are fundamentally critical. The interim water system and the coefficient of skillet essentially influenced the natural product to give up summer squash. Summer squash is delicate too and may be harmed from seed sowing to growing over the topsoil water. Since the depth of the establishment of summer squash is relatively shallow, soil water needs to be kept up to more than 65%

of the soil water capacity that can be reached to bypass and avoid water shortage. (Mario et al. 1997).

With the sub-water subsystem, the EC of the growth medium rises significantly quicker than with the drip irrigation frameworks. Fewer concentrated fertilizer arrangements should be used to keep the EC of the growth medium at the specified point to expect a decrease in *Cucurbita* surrender. Seed quality for squash edit generation is influenced by several indoor and natural components during seed progression (Araujo et al. 1982). The surrender and quality of squash seeds can be a move to sufficient dispersal of populace plants and the amount of dust that affects the quality of pollination and fertilization (Lima et al. 2003).

8.7.3 Tolerance for Environmental Stress

Summer squash is moderately sensitive to salinity stress (Blaylock 1994). The rootstocks consumption has increased offspring vigor via soil pathogen resistance and the resilience of *C. pepo* to moo soil temperatures or salted (Ruiz et al. 1997). The usage of rootstock is an effective approach to reduce sodium toxicity by increasing salt tolerance. Sevengor (2010) developed salt-tolerant genotypic variations among local squash and pumpkin genotypes in Turkey (Balkaya and Kandemir 2015). In many regions of Turkey, winter squash and pumpkin can grow on non-irrigated soil. Winter squash and pumpkin growth can also be considered an acceptable solution to the salinity or drought problem (Kurtar et al. 2016; Sevengor et al. 2011). Winter squash and pumpkins which can be cultivated without irrigation are a strong choice in arid and semiarid ecosystems for the soils with salinity problems. Many researchers are working on the abiotic stress tolerance of *C. pepo* (Table 8.5).

8.7.4 Genetic Improvement Objectives

Varieties of squash had a broad range of differences. Vegetative qualities and early yield are the most important traits in squash. Abdein (2016) analyzed seven yield and quality characteristics for *C. pepo*, as shown in Table 8.6. For all called characteristics, the heterosis versus mid-parents tended to be very significant values. The heterosis over mid-parents tended to be highly noteworthy values. For most of

Table 8.5 Abiotic stress tolerances of *Cucurbita pepo*

Tolerance type	References
Pollen water stress	Gay et al. (1987)
Drought and salt stress associated with mycorrhiza	Harris-Valle and Esqueda (2011)
Salt stress	Balkaya et al. (2016)
Low-temperature	Carvajal et al. (2018)

Prepared by A. A. Ibrahim

Table 8.6 The mean performances for yield and its components traits of seven squash varieties

Parents	Fruit length (cm)	Fruit diameter (cm)	Fruit shape index	Total soluble solid % (%)	Fruit weight (g)	Number of fruits per plant	Fruit yield per plant (g)
Parent 1	12.93	33.13	4.24	3.33	117.6	223.4	2.73
Parent 2	4.73 L	7.77 H	0.61 L	5.63 H	130.1	115.9	2.11 L
Parent 3	10.93	2.63 L	4.15	3.53	93.9 L	224.8	2.33
Parent 4	13.07 H	2.83	4.61 H	4.37	98.9	30.2 H	2.87
Parent 5	11.67	3.07	3.81	3.37	107.7	26.9	2.81
Parent 6	8.63	6.53	1.32	2.43 L	134.9 H	15.1 L	2.23
Parent 7	12.47	2.93	4.26	3.57	112.7	28.2	3.15 H

Where: H = The highest value L = The lowest value. Source: Abdein (2016)

the traits tested, the estimation of heterosis over the superior parent appeared of profound importance. None of the crosses showed the most extreme heterogeneity for all the characteristics. Still, for the different traits, the critical and alluring degree of heterosis over mid-parents and superior parents was found in a few crosses.

8.8 Germplasm Diversity and Conservation

8.8.1 Plant Germplasm Conservation

Germplasm conservation is a highly important and attainable technique for studying and genomic conservation of the most valuable crops. Germplasm contains entire genes of this species and can preserve this information for a long time which can be used in the future. To conserve genetic information for new species, endangered species and unidentified species, germplasm has been put in the genebank. Gene libraries or genebanks can assist in vitro in evaluating germplasm genetic variation (transgene) and in selecting the best germplasm (Reitsma et al. 2014).

For the first generations, genetic diversity may be influenced by the loss of genetic information, so germplasm is used as a success factor to retain genetic information. Genetic diversity may be lost due to climate change variability, hybrid use and enhanced landraces. Genebanks have a crucial role in the perseverance of landraces and alleles and can be used for the genetic conservation of valuable and economic crops afterward. The conservation of germplasm includes cultivated plants, commercial genotypes or landraces and breeding lines (Byrne et al. 2018; Cruz-Cruz et al. 2013).

8.8.2 In-situ Conservation

Squash genetic resources are the natural genetic materials for squash breeding and a source of continued growth in production, biotic resistance, abiotic tolerance, yield and its components and quality traits improvement. Genetic erosion has been recorded in the crib regions of yield domestication, where the loss of traditional cultivars parallels the specialization and intensification with which new high-yielding varieties are introduced and disseminated (FAO 1996; Hammer and Teklu 2008). In situ preservation represents one form of preservation of crop genetic resources, which means conserving genetic resources in natural environments (Brush 1995; Nabhan 1985; Maxted et al. 2013). Maintaining the genetic diversity of crops in farmers' fields, in in-situ, is important to preserve crop breeding gains and provide the possibility of further development in the future. The genetic conservation of species using natural individuals in biosphere reserves such as national parks as conserve props in their natural habitat (Spataro and Negri 2013). Research models on the potential effect of climate change on pumpkin allocation indicate that all pumpkin distributions are expected to decline dramatically over the next 60 years. (Lira et al. 2009).

8.8.3 Ex-situ Preservation

Preservation of genetic information for critical species (endangered species, rare species, and national crops outside its natural habitats. This conservation can be preserved species by using different methods such as in vitro seeds and stored in the genebank for a long time till use (Engelmann 2004). Large collections of *cucurbits* are preserved in Genebanks worldwide (Bolivia, Brazil, Colombia, Costa Rica, Czech Republic, Italy, Mexico, Portugal, Russia, Spain, Turkey and USA) (Clark et al. 1991; Diez et al. 2002; Ferriol and Pico 2008; Karlova 2008; Lebeda et al. 2007; Nuez et al. 2000). Restricted funding for Genebank activities may limit the restoration efforts required to make collections accessible to breeders for distribution. A huge amount of ex-situ *Cucurbita* germplasm was collected and maintained across the globe in various agricultural centers. These centers and the international consortium for squash genetic resources in Europe, the USA, Africa, Asia and Australia. A significant proportion of *Cucurbita* is kept in the U.S. national plant germplasm network (<https://npgsweb.ars-grin.gov/gringlobal/search.aspx>).

8.8.4 Cryopreservation

The cells or tissues used in this process are stored in a frozen state. That occurs by using very low temperatures, using solid CO₂ (−79 °C). Usage also of nitrogen vapor (−150 °C) and liquid nitrogen vapor (−196 °C) (Karthi and Engelmann 1994). This process involves multiple stages such as freezing, thawing and re-culturing. This freezing temperature can preserve the cells in inactive form so

Table 8.7 Listing of valuable genebanks for squash genetic resources

Genebank	Country	Website
Institute of Crop Science, Chinese Academy of Agricultural Sciences	China	http://www.cgris.net/photobase/default.html
Czech Republic Gene Bank	Czech Republic	https://grinczech.vurv.cz/gringlobal/search.aspx
Leibniz Institut für Pflanzengenetik und Kulturpflanzen forschung (IPK)	Germany	https://www.ipk-gatersleben.de/en/gbisipk-gaterslebendebis-i/
Leibniz-Institut für Pflanzengenetik und Kulturpflanzen forschung (IPK)	Europe	https://eurisco.ipk-gatersleben.de/apex/f?p=103:4:::NO:RP:P4_NATIONAL_INVENTORY:3
Nordic Genetic Resource Centre, NordGen	Sweden	https://sesto.nordgen.org/sesto/index.php?thm=sesto
USA Plant Germplasm Introduction and Testing Research Station, Pullman	USA	https://npgsweb.ars-grin.gov/gringlobal/search.aspx

that they can be saved for a long time (Jang et al. 2017; Kartha 1981). The cryopreservation method can be used as embryos, meristems, ovules and seeds on any tissue from the crop. Specific compounds such as dimethyl sulfoxide, glycerol, ethylene, propylene, sucrose, mannose and glucose can be used to protect the cells from damage (Kaviani 2011). Table 8.7 shows a list of the most significant gene banks in the world for preserving the genetic resources of squash.

8.8.5 Cytogenetics

Basic chromosome number in genus in the genus *Cucurbita* ($2n = 2x = 40$) (Domblides et al. 2018; Gong et al. 2013). Whitaker and Flory (1955) and Koxyxob (1925) identified several $2n$ chromosomes $2n = 40$ and $2n = 44-48$, respectively. Therefore, there has been a misunderstanding of the exact number of $2n$ chromosomes in such varieties until now. Functional genome regions, variation in structure as an inversion and translocation between genome regions can be revealed by similarities and differentiation between the genomes of different *Cucurbita* (Chaney et al. 2016; Morrell et al. 2012). The chromosome size is very small (Fig. 8.7). The numbers of chromosomes of different *C. pepo* cultivars vary according to heredity. The morphology of chromosomes is undifferentiated where, in addition to the chromosome, a long arm or short arm of individual chromosomes may not be identified as small spots from each other, so it is difficult to estimate karyotype (Weeden 1984; Weeden and Robinson 1990).

Flow cytometry provided strong knowledge about the *C. pepo* haploid genome was about 500 million base pairs long (Arumuganathan and Earle 1991). The nucleus of the vegetative cell *C. pepo* ($2n$) contains about 1.04–1.08 pg of DNA. Because of the morphological characteristics of this crop, several molecular markers have been utilized to study the genetic map (Havey et al. 1998). To monitor the

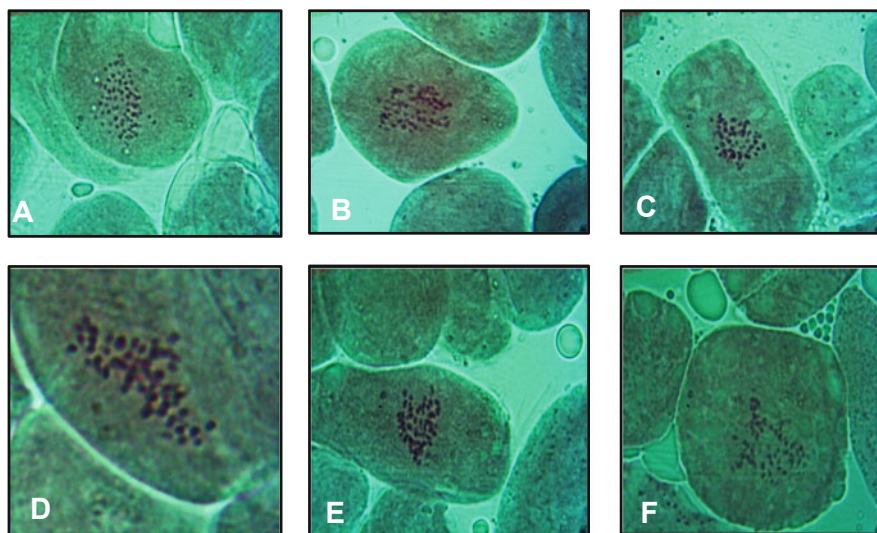


Fig. 8.7 Chromosome number of various *Cucurbita pepo* cultivars. (a–c) Chromosome number of *C. pepo* (Eskandarani) and (d–f) Chromosome number of *C. pepo* (Coppi). (Source: Photo by A. A. Ibrahim)

genetic variability, self-pollination for four consecutive generations of *C. pepo* inbreeding depression was performed. The self-pollination ratio indicated a range from 0.16 to 0.54, and this result varies by character, year and condition (Hayes et al. 2005). Whitaker and Robinson (1986) suggested that the response of different types of cucurbits to inbreeding could reflect these different types of pollination. Variations in *C. pepo* gene sequences resulted in variations in phenotypes that assisted in successful landrace selection. Gene and allele-specific markers are related rather than random markers to the appropriate genes.

8.9 Traditional Breeding of Summer Squash

Inter-species hybridization is utilized to enhance yields by imparting certain attributes from their wild relatives. (Bowley and Taylor 1987). In the *Cucurbita* genus, there have been a few challenges in granting interspecific crossbreeds between five developed species (*C. maxima*, *C. pepo*, *C. moschata*, *C. ficifolia* and *C. argyrosperma*) and some wild species. The success of hybridization is dependent on species within this genus. Curtis (1940) recorded that the summer squash F_1 has been observed to produce female flowers 10 days earlier than either parent. He added that more female flowers were produced by F_1 than either parent.

Hutchins and Croston (1941) also found that winter squash *C. maxima*, the first female flower in F_1 opened much earlier than their parents.



Fig. 8.8 Four squash *Cucurbita pepo* varieties. Eskandarani (Parent 1), Zucchini Mezza Lung Bianco (Parent 2), White Bush Scallop (Parent 3) and Zucchini Nano Verde di Milano (Parent 4). (Source: Abdein 2005)

Table 8.8 The parent genotypes and their origins

No.	Parent name	Origin
1	Eskandarani	Egypt
2	Zucca Patisson custard white	France
3	All Green Bush	United Kingdom (UK)
4	Courgette Orelia	Germany
5	Sakiz	Turkey
6	Copi	Egypt
7	Gapla	Syria

Source: Abdein (2016)

Abdein (2005) has studied the nature of heterosis and evaluated the genetic behavior of certain quantitative squash traits, demonstrating the genetic materials utilized to study genetic diversity along with the different genotypes of squash. Abd El-Hadi et al. (2014) and Abdein (2016) considered the species to have a place in the genetic materials of seven squash lineages. The following parent lines are Eskandarani (P1), Zucca Patisson custard white (P2), All Green Bush (P3), Courgette Orelia (P4), Sakiz (P5), Copi (P6) and Gapla (P7) (Fig. 8.8). Seeds of these lines were taken from diverse origins, as shown in Table 8.8. Heterosis for some squash economic traits was estimated (Abdein 2016; Abd El-Hadi et al. 2005; Al-Araby 2010; Al-Ballat 2008). For both yield and its components traits, they observed that heterosis was detected only over the mid-parents.

8.9.1 Inbreeding Depression and Selection

Three generations of inbreeding with selection were used to improve the summer squash variety Eskandarani. Self-pollination can be utilized in reproductive species of the genus *Cucurbita*, although the plants interbreed, there is almost no loss of durability due to inbreeding. (Allard 1971; Robinson 2008; Whitaker and Robinson 1986). Selection is a methodology that allows again in quantitative traits inheritance, resulting in a new population than the original, as much as on the average performance of the best individuals (Fehr 1987).

Abd El-Al and Khalaf-Allah (1973) perused inbreeding depression (ID) in the summer squash variety Eskandarani. They noted that early yield inbreeding depression and the number of fruits per plant appeared in the F_2 generations. They perused the deviation of F_2 generations against F_1 hybrids and reported the inbreeding depression manifestation for stem length in F_2 generations.

Borghi et al. (1973) studied inbreeding depression of the anthesis day of the earliest female flower. Results confirmed that inbreeding affects *Cucurbita* species.

In another study, Schuster et al. (1974) recorded that the total fruit yield decreased by 96% relative to open-pollinated plants after 11 generations of self-pollinated in the variety Giessener. In this sense, Hassanein et al. (1975) noted that local variations in summer squash differed in their response to inbreeding concerning stem length and the number of plant leaves. They clarified that no detectable deviations between summer squash inbred lines and their ancestral parents regarding yield per plant.

El-Gazar (1981) recorded significant inbreeding depression with values of 39.27, 84.03 and 83.45% for fruits number per plot, fruit weight for each story, and fruit weight per plant, respectively. Metwally (1985) recorded highly significant inbreeding depression for total yield in squash. The results showed that ID values for each plot's number of fruits and fruit weight per plot were 17.7 and 19.7%, respectively. He registered various ID values as follows -7.0, -1.41, -2.31 and -3.61% of the nodes for the first female flower, date of birth of the first male flower, and the first date of forming a female flower and the number of male flowers per plant, respectively. In their investigation of *C. pepo*, El-Diasty and Kash (1989) stated that the ID values were 11.91, 37.71 and 9.91% for the leaves number, the male flowers number and the stem length, respectively.

Within 10 years in *C. Pepo*, El-Gendy (1999) determined ID values for plant length, number of plant leaves, first female flowering nodes' number, number of male flowers per plant and time of the first female flower as follows 0.162, -17.11, 1.98, -7.24 and 6.73%, respectively for F_2 generations. She estimated ID in squash and recorded that the estimates were as follows -21.49, 20.97, -1.71, -2.49 and -3.98% for the plant fruits number' weight, the fruit length, the fruit thickness and the fruit shape index.

Abd El-Hadi et al. (2004) and Gabr (2003) suggested that most studied traits of ID (F_1 , F_2), ID (F_{1r} , F_{2r}) and ID ($F_{1,1r}$, $F_{2, 2r}$) had inbreeding depression (Fig. 8.9). For those traits which had large estimates of heterosis, the estimated values were greater. The performances of all F_2 generations ($F_{2, 2r}$) were better than the parental varieties not only for vegetative traits but also for yield and its components traits. Mohan et al.



Fig. 8.9 Inbreeding depression in squash F_2 generations ($P_3^{\text{♀}} \times P_4^{\text{♂}}$). (Source: Gabr 2003)

(2012) and Xanthopoulou et al. (2019) found significance in all crosses for most of the vegetative traits in ash gourd. Concerning earliness traits, all crosses showed significant inbreeding depression for all traits except the number of days to first flowering in crosses 2 and 3 and the days are taken for first fruit production in cross two.

8.9.2 Heritability

El-Gazar (1981) found that the narrow-sense heritability was 53.91, 42.50, 31.59 and -32.92% for stem height, the number of plant leaves, the number of male flowers and the number of first female flowering nodes, respectively. Also, the broad-sense heritability was 48.06, 67.37, 94.75 and 10.44% for the same traits, respectively. In squash, El-Gazar and Zaghloul (1983) found that heritability values were moderate and ranged from 50 to 63% for the node position of the first female flower. Metwally (1985) recorded high heritability values in a broad sense for the number of the plant's male flowers and the number of days to the first female flowering.

Abd El-Maksoud (1986) investigated 20 F_1 hybrids including five varieties of summer squash. In both wide and narrow sense values, he calculated the heritability for virgin traits like the number of nodes for the first female flower, the number of each plot's fruits in the first seven selections and the fruits' weight per plot in the first seven picks were 59.94, 97.4 and 45.85%. El-Shawaf et al. (1986) used a 4×4

partial diallel cross to test heritability for yield and its constituents. They concluded that heritability estimations in the wide-ranging sense ($h^2_b\%$) were above average for all traits except yield and its components traits in the limited sense ($h^2_n\%$).

Metwally et al. (1988) used partial diallel crosses (5×5) to estimate heritability in the seed yield and its associated characteristics in the summer squash. They found that d that heritability assessments in a narrow sense were mild for seed index and mean length of ripe fruit of 44.3% and 35.0%, respectively. El-Diasty and Kash (1989) found a heritability of a narrow-sense ranging from 15.59 to 31.58% for the plant fruits' number and the fruit weight.

Dahiya et al. (1990) revealed that plant height showed high heritability values. The maximum rate in the broad sense was 97.64% for the fruit diameter, while it was 87.93% in the narrow sense.

Abd El-Hadi et al. (2004, 2005) found that heritability in the wide-ranging sense ($h^2_b\%$) was more significant than the narrow sense heritability ($h^2_n\%$). For the first picking date (1stPD), the approximate heritability values in a limited context ($h^2_n\%$) ranged from 0.00 to 60.38%.

Al-Jebory (2008) used a co-mating project of six female strains \times two male lines to produce 12 first-generation hybrids. To determine heritability, all genotypes of vegetative traits were evaluated r. Fayeun et al. (2012) recorded a high predicted genetic gain in vine height, leaf's fresh weight, vine weight, the leaves number and viable yield. Broad-sense heritability for vegetative and qualitative characteristics was examined in crossbreeds from fruit-type groups in *C. pepo* and *C. moschata* (Darrudi et al. 2018; Davoodi et al. 2016).

8.9.3 Genotypic and Phenotypic Correlation

Total marketable fruit weight per plant was significantly correlated with the total fruit weight. The recurrence dissemination polygons for an open-pollinated summer squash called Eskandarani recommended the major gene(s) with a calculable natural impact that controls the blossom sex expression (Brewbaker 1964; Mohamed 1996). Latent qualities appeared to have an impact on the propensity to gentility, number of fruits per plant and earliness (El-Tahawy 2007).

Abd El-Maksoud et al. (2003) and Abdein (2005) found that most sets of traits showed a negative genetic and phenotypic correlation. However, the following pairs of features exhibited positive correlation coefficients such as sex percentage, times to the first female flower head, prematurely yield in terms of weight and number of fruits in 7 harvests per plant. They also added that a breeding program system to improve one or more of these characteristics must enhance the others. Abdel Sayyed et al. (2003), in sweet melon, assessed correlations between the fruit's quality characteristics. They found flesh texture to be negatively associated with flesh flavor and aroma, while positive genotype associations between fleshy flavors were observed. Al-Jebory (2006) studied genotypic, phenotypic and environmental correlations for several characters of summer squash. Sanín et al. (2015) used

open-pollinated introductions to study the genetic correlation, relationship and path analysis for the squash yield and fruit components of diallel crosses.

8.10 Molecular Breeding

8.10.1 SDS-PAGE Electrophoresis

Choudhary and Ram (2000) described 65 strains of melon germplasm using a seed protein extract. A total of 15 seed protein bands that were supplied into four different zones A, B, C and D could be resolved. Banding patterns in C and D zones were not polymorphic.

Using a single seed, Singh and Ram (2001) suggested that SDS-PAGE has examined seed storage protein of the cucumber genotypes under reduced conditions. Seed proteins consisting of 19 germplasm genotypes were expressed in a total of 17 domains divided into 3 regions (A, B and C), A region containing six bands, region B had seven bands and region C had four bands. The 19 lines can be divided into eight groups, based on protein profiles. Therefore, some lines of germplasm may be differentiated on protein profile bases.

Abd El-Salam et al. (2010) tested five snake cucumber genotypes. The four genotypes showed band patterns differing. The variety Ismailia 2 provided the highest number of six bands, and the minimum number of three bands was present in the Fayoom 5 and Kalyoubia varieties. On squash, El-Shamy (2009) showed protein pattern variation and content of stable and cucumber mosaic virus (CMV) symptoms infected squash leaves using SDS-PAGE.

Al-Tamimi and Attyaf (2014) applied SDS-PAGE techniques to fingerprint seven squash genotypes. SDS electrophoresis pattern of total seed protein demonstrated low polymorphism due to four monomorphic bands from six bands produced. SDS electrophoresis technique produced 33.3%, polymorphic bands.

Abdein (2016) discriminated against biochemical fingerprints using the protein electrophoresis technique for squash leaves. Protein electrophoresis successfully produced reproducible polymorphic bands. The profiles produced showed high polymorphism among studied parents.

8.10.2 Molecular Marker-assisted Breeding

Molecular markers of DNA such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and inter simple sequence repeats (ISSRs) have already been evaluated for diversity within *C. pepo* (Dalda-Şekerci et al. 2020; Esteras et al. 2011; Lebeda et al. 2007). Much of the opinions focused on determining the genetic and developmental relations between the wild-tamed species, between the two subspecies and between the cultivar bunch, counting as many European landrace agents (Ferriol et al. 2003; Paris et al. 2003).

Despite the growing economic importance of squash varieties for seed production, no information is available on population structure within and between accessions with modified seed coats and the range of inbreeding found in those accessions is not available. Earlier genomic studies were based primarily on intra-species diversity within the genus *Cucurbita* or on genetic diversity between subspecies *C. Pepo*, focusing on certain groups of cultivars (Ferriol et al. 2003; Formisano et al. 2012; Gong et al. 2008, 2012; Paris et al. 2003).

Using random amplified polymorphic DNA (RAPD) markers to analyze genetic variants in cucurbits has been widespread and more effective (Cuevas-Marrero and Wessel 2008; Ntulia et al. 2015). Previously, genetic variation within *C. pepo* was evaluated using allozyme and various DNA marker systems and inter-simple sequence repeats (ISSRs) (Dalda-Şekerci et al. 2020; Esteras et al. 2011; Lebeda et al. 2007). It is widely known that, at the level of plant genomes, using DNA-based markers is very effective in assessing genetic diversity and identifying plant accessions. Inter simple sequence repeats (ISSR) markers are easy-to-use, PCR. Polymorphic random amplified polymorphic DNA (RAPD) is used to verify unique genetic marks and differences in a wide variety of plants such as the genus *Cucurbita* (Heikal and Hadia 2008). Mohamed et al. (2018) explored squash landraces that compete with RAPD markers to further assess genetic relations and similarity among squash landraces, established and assessed these genetic constitutions under Egyptian conditions in an exposed field experiment and finally stated that these molecular markers can be applied to classify squash landraces in molecular breeding. RAPD markers are thus valuable for assessing genetic variation within and between squash species.

El-Adl et al. (2012) used 21 RAPD primers, which produced high polymorphism levels among the seven cultivars of summer squash. Heikal and Hadia (2008) examined two PCR molecular marker strategies, arbitrary opening of polymorphic DNA (RAPD) and connecting basic grouping rehashes (ISSR) to clarify the polymorphisms and the connections between 14 landraces with three distinctive *Cucurbita* species (*C. pepo*, *C. moschata* and *C. maxima*).

Using various molecular markers, for example, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), SRAP (sequence-related amplified polymorphism), ISSR (inter simple sequence repeat) and AFLP (amplified fragment length polymorphism), molecular markers were embraced to consider an inherited variety in and among a few species of *Cucurbita* (Baranek et al. 2000; Dalda-Şekerci et al. 2020; Gwanama et al. 2000; Katzir et al. 1998, 2000; Montes-Hernandez and Eguiarte 2002; Ramon et al. 1991).

Michael et al. (2019) used 39 simple repeats of sequences (SSRs) to explain *C. pepo* genetic relationship among resistance accessions. Some researchers have utilized various molecular markers to study genetic variation among *C. pepo* (Table 8.9).

Table 8.9 Different DNA markers used in the genetic diversity of *Cucurbita pepo*

Molecular markers	No of primers	References
Simple sequence repeats (SSRs)	27	Formisano et al. (2012)
	18	Murovec (2015)
	10	Ntulua et al. (2015)
Random amplified polymorphic DNA (RAPD)	9	Ntulua et al. (2015)
	7	Tsivelikas et al. (2009)
	6	Heikal and Hadia (2008)
	5	Khalil and Hassan (2013)
	20	Santos et al. (2012)
	5	Mohamed et al. (2018)
	Inter Simple Sequence Repeat (ISSR)	7
	5	Khalil and Hassan (2013)
	5	Abdein (2018)
	15	Santos et al. (2012)
	6	Xanthopoulou et al. (2015)
Start Codon Targeted (SCoT)	6	Abdein (2018)
	7	Xanthopoulou et al. (2015)
Single-nucleotide polymorphism (SNP)	10	Xanthopoulou et al. (2019)

8.10.3 Genomic Resources

Genomic resources include numerous tools such as molecular markers, genotyping-by-sequencing (GBS), and genome-wide association studies (GWAS) that prioritize the establishment of single nucleotide polymorphism (SNP) that monitors economic traits. *C. pepo* was analyzed using the simple sequence repeats (SSRs) molecular marker and confirmed that *C. pepo* had the greatest genetic diversity in the Cucurbitaceae family. In *C. pepo*, genomic tools were improved using SNP to detect quantitative trait loci (QTLs). The *C. pepo* subsp. *pepo* genome morphotype was about 93% of the genome size and divided into 20 pseudo chromosomes. Xanthopoulou et al. (2019) recorded full *C. pepo* subsp. *pepo* and subsp. *ovifera* genome distinguished by various SNPs (Fig. 8.10).

Dense Dark genetic maps are important implements for effective molecular breeding. Several gene binding maps were generated in *Cucurbita*. The *Cucurbita* binding maps have been established in recent decades using both interspecific *C. pepo* × *C. moschata* and intraspecific crosses *C. pepo* ssp. *pepo* × ssp. *pepo* and *C. pepo* ssp. *pepo* × ssp. *ovifera* the related populations. Such maps were first constructed using randomly generated DNA amplification polymorphic (RAPD) dominant markers and amplification polymorphism (AFLP) (Brown and Myers 2002; Lee et al. 1995; Zraidi et al. 2007), and SSRs were completed (Gong et al. 2008, 2012). Zucchini and scallop genotypes were utilized to produce the *C. pepo* transcriptome and were earlier used as parents of the F₂ mapping group which was used to create the first SNP-based gene map and map of quantitative trait loci (QTLs), flower and fruit characteristics (Esteras et al. 2012).

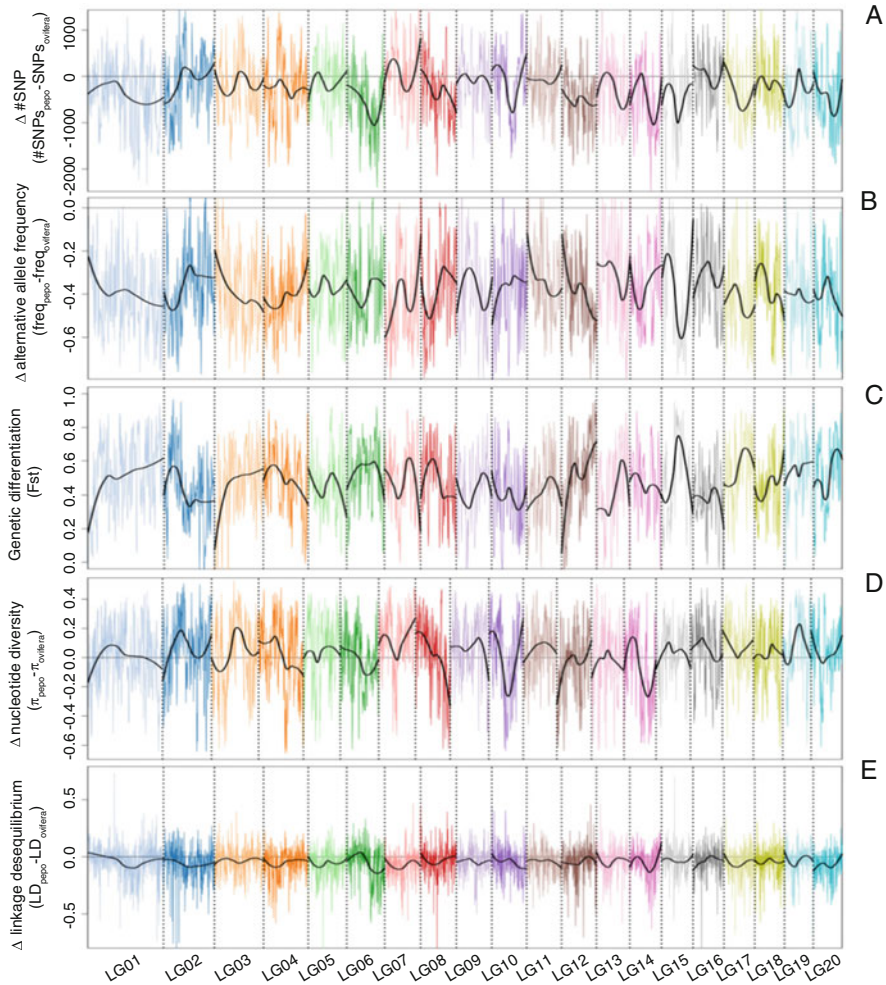


Fig. 8.10 Broad genome variations between *Cucurbita pepo* subsp. *pepo* and subsp. *ovifera*. (a) Variations in SNPs, (b) Frequency of the alternative allele (allele doses not present in the reference genome), (c) genetic differentiation, (d) nucleotide diversity and (e) linkage disequilibrium between *Cucurbita pepo* subsp. *pepo* and subsp. *ovifera* along the chromosomes. Vertical dashed lines indicate the end/beginning of a chromosome. The solid horizontal line marks the absence of differences. (Source: Xanthopoulou et al. 2019)

Esteras et al. (2012) formed a saturated gene map based on 315 high-quality markers (304 SNPs and 11 SSRs) from a *C. pepo* subsp. *pepo* var. Zucchini MU-CU-16 \times *C. pepo* subsp. *ovifera* var. Scallop UPV-196 cross (Fig. 8.11). Also, Montero-Pau et al. (2017) constructed a saturated genetic map based on genotyping-by-sequencing from a Zucchini \times Scallop (ssp. *pepo* \times ssp. *ovifera*) inter-sub-specific cross. Several thousand SNP markers were detected and

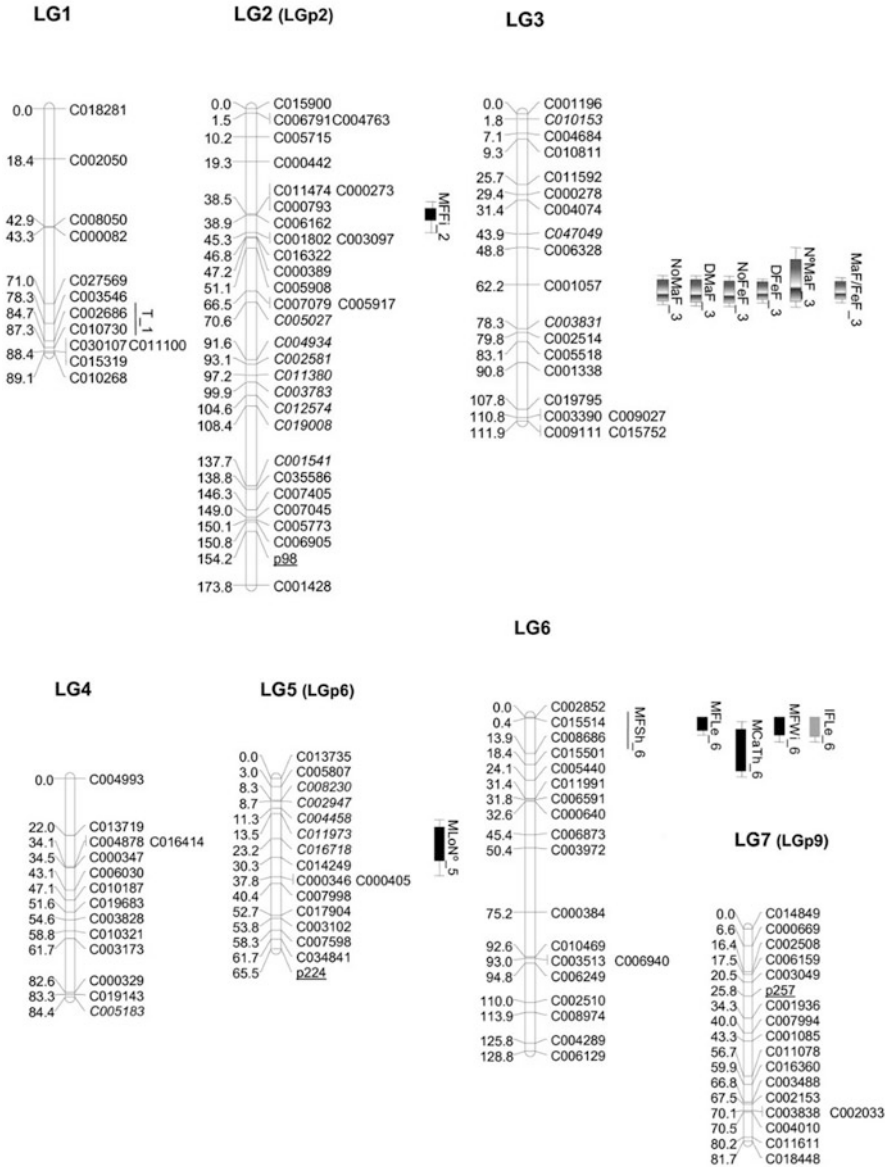


Fig. 8.11 Genetic map of Zucchini \times Scallop F_2 population. Linkage map and locations of quantitative trait loci (QTL) whose impacts have been validated in the backcross populations linked with vine improvement, flowering and fruit quality based on 146 F_2 plants resulting from a Zucchini \times Scallop cross. QTL indicated in light grey, grey or black correspond to flowering and immature mature fruit traits, respectively. (Source: Esteras et al. 2012)

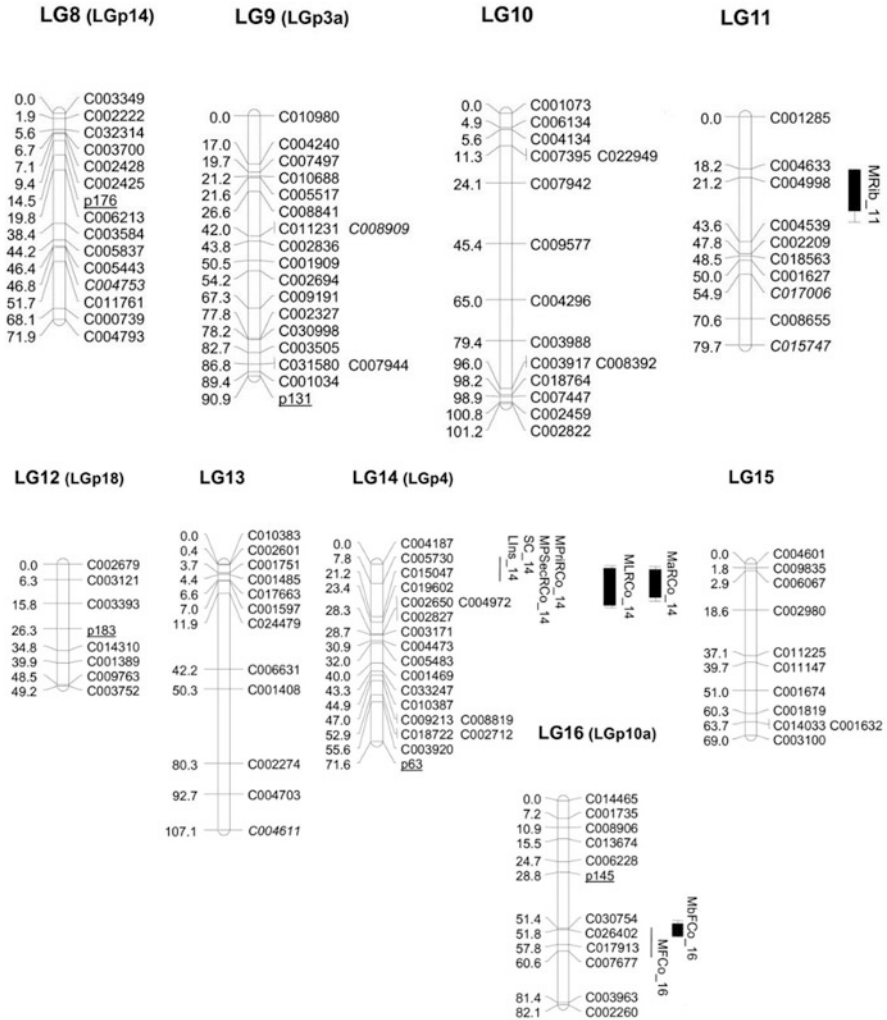


Fig. 8.11 (continued)

genotyped, supported by the creation of a high-density linkage map based on 7718 SNPs (averaging 386 markers per binding group) covering 2817.6 cm of the entire genome, which is a significant improvement compared to earlier maps. Especially different mapping populations were used to map Quantitative and qualitative characteristics (Esteras et al. 2012), Montero-Pau et al. 2017; Zhong et al. 2017), dwarf trait (Xiang and Duan 2018), resistance to tomato leaf curl New Delhi virus (Sáez et al. 2020), fruit and agronomic traits (Del Valle Echevarria et al. 2020) and seed traits (Wang et al. 2020). In *Cucurbita* spp. much QTL mapping was performed utilizing several types of DNA markers. Some QTL mapping for the various squash

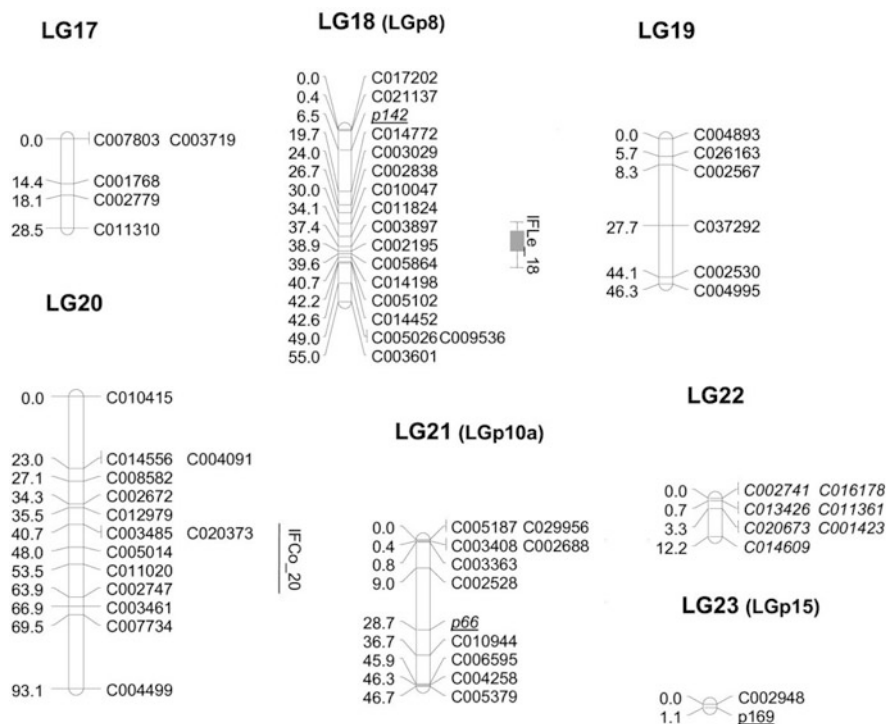


Fig. 8.11 (continued)

characteristics is shown in Table 8.10. Using QTL mapping speeds up squash breeding as it makes it easier for the squash breeder to a) identify specific genes that control the quality, b) understand the impact of genes/QTLs that control traits, c) determine the gene/QTL location and d) screen of relationships between the diverse genes/QTL of attention. Each of these goals should improve the pyramid of various target genes into one genotype and reflect the variability of the examined germplasm.

8.11 Tissue Culture Applications

Advancing recovery engineering for in vitro culture is part of management development. In a few species of the cucurbit family, recovery by physical embryogenesis is limited (Debeaujon and Branchard 1993). Embryogenesis is the one that was a reliably established form of recovery for *C. pepo* (Chee 1991, 1992; Gonsalves et al. 1995; Jelaska 1972, 1974). Indirect organogenesis is a pathway to recovery, reported in summer squash *C. pepo* (Pal et al. 2007).

Induction of gynogenesis by culturing unfertilized ovary/ovule is an efficient way to speed up breeding programs by getting homozygous materials in a short period,

Table 8.10 Listing of various kinds of DNA markers identified for QTL in *Cucurbita* spp.

Trait	Mapping population	QTLs	Marker	Reference
Quantitative and qualitative traits	F ₂ individual	59 QTL	Single nucleotide polymorphisms (SNPs)	Esteras et al. (2012)
Fruit-related traits	F ₂ individual	29 QTLs	Single nucleotide polymorphisms (SNPs)	Zhong et al. (2017)
Fruit-related traits	Recombinant inbred lines (RILs)	48 QTLs	Genotyping-by-sequencing	Montero-Pau et al. (2017)
Fruit-Associated	Recombinant inbred lines (RILs)	QTLs	Simple-sequence repeats (SSRs), Single nucleotide polymorphisms (SNPs) and diversity array technology (DArT)	Kaźmińska et al. (2020)
Dwarf trait	F ₂ individual	3 QTLs	Expressed sequence tags (EST-SSRs) and Simple-sequence repeats (SSRs)	Xiang and Duan (2018)
Resistance to tomato leaf curl New Delhi virus	F ₂ individual	Major QTL	Single nucleotide polymorphisms (SNPs)	Sáez et al. (2020)
Fruit and agronomic traits	Backcross (BC1)	QTLs	Single nucleotide polymorphisms (SNPs)	Del Valle Echevarria et al. (2020)
Seed traits	F ₂ individual	10 QTLs	Specific length amplified fragment (SLAF)	Wang et al. (2020)

Prepared by Prof. Dr. K.F.M. Salem

and significant advances have been made in recent years with the culture of cucurbitaceous crops (Min et al. 2016). Shalaby (2007) investigated the impact of genotype, female flowers' position on the stem of a plant, temperature and sucrose concentration on induction gynogenesis of squash *in vitro* and stated that genotype is the main factor affecting *in vitro* reproduction in squash.

The highest ratio of ovules reacting, and the number of plantlets per plate was found in the second female. Incubated ovules developed a better embryogenic response at 4 or 32 °C for 4 days. The significant result was obtained from the ovules in the MS culture medium of Murashige and Skoog (1962) containing 30 g/L of sucrose. Zou et al. (2020) observed that induction levels for both embryo-like structures (ELs) and plantlets differed for different lines present in the same culture conditions, and various lines had various optimal initiation media. Genotype and initiation media may have important impacts on ELS initiation, but they performed a more significant role in their interaction.

Kathiravan et al. (2006) investigated the cultivation and propagation of *C. Pepo* *in vitro* through direct organogenesis from cotyledons. They noted that, in addition to the impact of ploidy in cucurbit populations, a close association exists between the

propagation of chimeric plants and seed measurements correlating with the medium composition.

Previously, plant regeneration of the *Cucurbita* was reported through direct and indirect organogenesis. The development of somatic embryos in six squash *C. pepo* cultivars was also recorded by Carol et al. (1995) using cotyledon segments. Similarly, cotyledons may be utilized for the regeneration of squashes *C. pepo* and *C. maxima* through direct organogenesis (Ananthakrishnan et al. 2003).

Kintzios et al. (2002) stated that the use of 2,4-dichlorophenoxyacetic acid (2,4-D) for 24–48 h in the treatment of *C. pepo* leaf improved plant regeneration and growth by 143% and proposed that this approach be used in the regeneration of *C. pepo* from embryos.

A few molecular markers play critical roles in the study of clonal dedication and among them, start codon targeted (SCoT) and randomly amplified polymorphic DNA (RAPD) markers were commonly applied (Rathore and Rai 2014). *C. pepo* embryogenic callus is done on media complemented with one of a few manufactured auxins (Jelaska 1974). Choosing adjusted sublimes from long-term squash societies which developed in an auxin-free medium was conceivable (Krsnik-Rasol et al. 1982). Figure 8.12 occurs in distinctive stages of *C. pepo* Egyptian cultivar Eskandarani tissue culture (Badawi et al. 2008).

8.12 Genetic Engineering and Gene Editing

Genetic engineering is an effective method used to recombine DNA technology in the improvement of new genotypes resistant to many diseases. Production of new genotypes had particular genes as a result of the combination of millions of crosses-translation of undesirable genes with beneficial genes through conventional breeding. Often, the loss of one desired gene through the rearrangement of parent genes during the crossing (Danida 2002).

Conventional breeding took place through exchanges between very closely related plant species and results from unwanted genes during gene transfer, and this process takes a long time to achieve the best result. The genetic engineering method is capable of transferring one or two genes, at least among related or distant organisms. Genetic engineering assists in definite time crop improvement by removing or switching undesirable genes (Lopez-Bucio et al. 2000). There are about 70 genes reported from *C. pepo*, 30 for *C. moschata* and 19 for *C. maxima* (Paris and Padley 2014).

The genetic transformation was used in *C. pepo* for virus-resistant (Clough and Hamm 1995; Collinge et al. 2010; Fuchs et al. 1998; Gaba et al. 2004; Tricoli et al. 2002). Genetically engineered zucchini and crookneck squash with resistance to cucumber mosaic virus, watermelon mosaic virus, and yellow zucchini mosaic virus has been widely commercially distributed in the United States (Fuchs and Gonsalves 2007). Resistances are originated from genes that code for the virus envelope proteins that are incorporated into the squash genome. Genes like these can be introduced into elite breeding lines and recombined with other attributes.

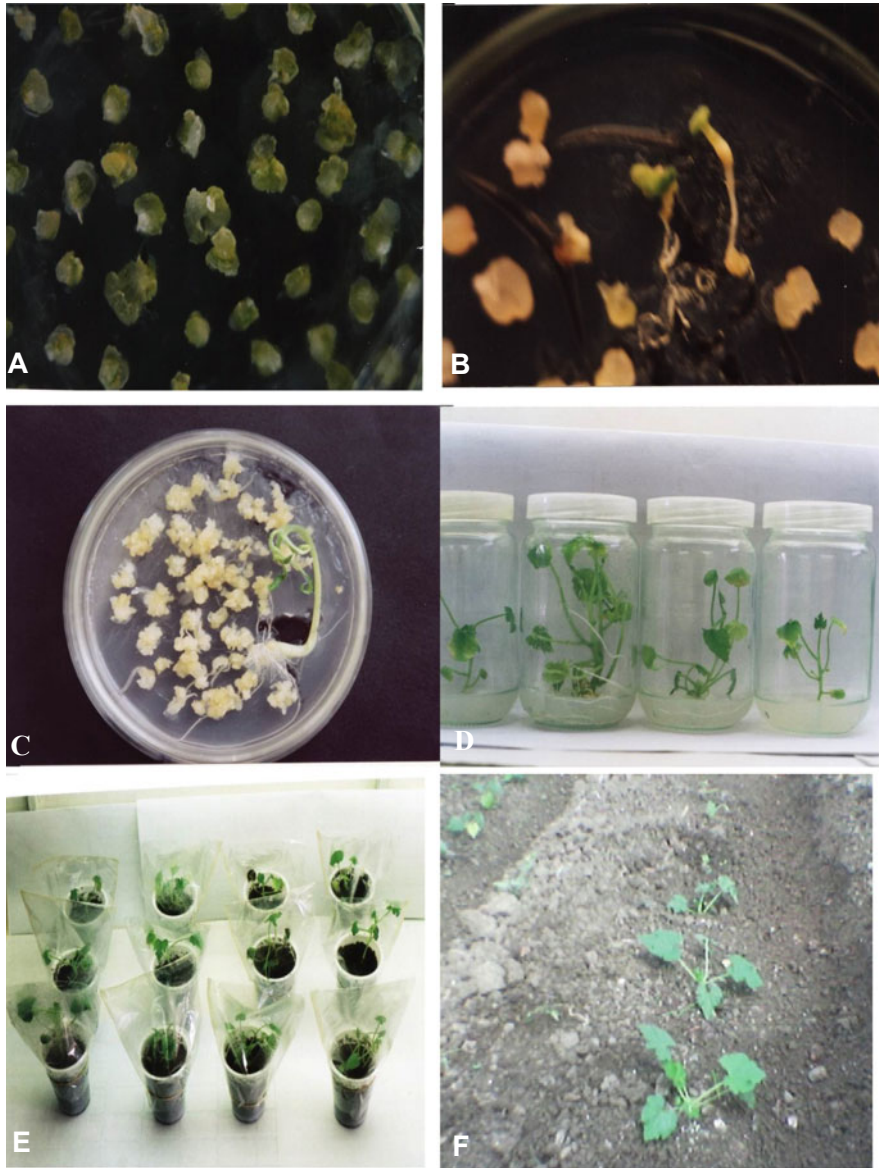


Fig. 8.12 Different phases of ovules culture in squash by tissue culture. (a–c) propagated plant from an ovule, (d) propagated plant with storage root, (e) propagated plant with a plastic bag and (f) plant transferred to soil. (Source: Badawi et al. 2008)

Very high rates of resistance to viruses have been found, in both artificial inoculation trials and the field (Fuchs et al. 1998). The appearance of recent viruses causing heavy losses on cucurbit are emerging every few years, genetic modification

techniques can provide an economically viable way to counter them if they are relatively cheap.

Recently, a new type of genome-editing technology has been established, namely the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) method. The CRISPR/Cas system theory was developed from a prokaryotic immune system adaptive to the type II organism (Jinek et al. 2012). They were first recognized as an unusual sequence feature in the *Escherichia coli* genome in 1987, comprising of a series of 29 nucleotide repeats separated by distinct 32 nucleotide spacer sequences (Ishino et al. 1987; Wiedenheft et al. 2012). Later, repeated sequences with similar interval repeat patterns were established in the genomes of bacteria and archaea, but the functions of these repeats were not clear until 2005 when three separate studies observed that the spacer sequences were identical to a portion of the virus and plasmid sequencing (Bolotin et al. 2005; Mojica et al. 2005; Pourcel et al. 2005) (Fig. 8.13).

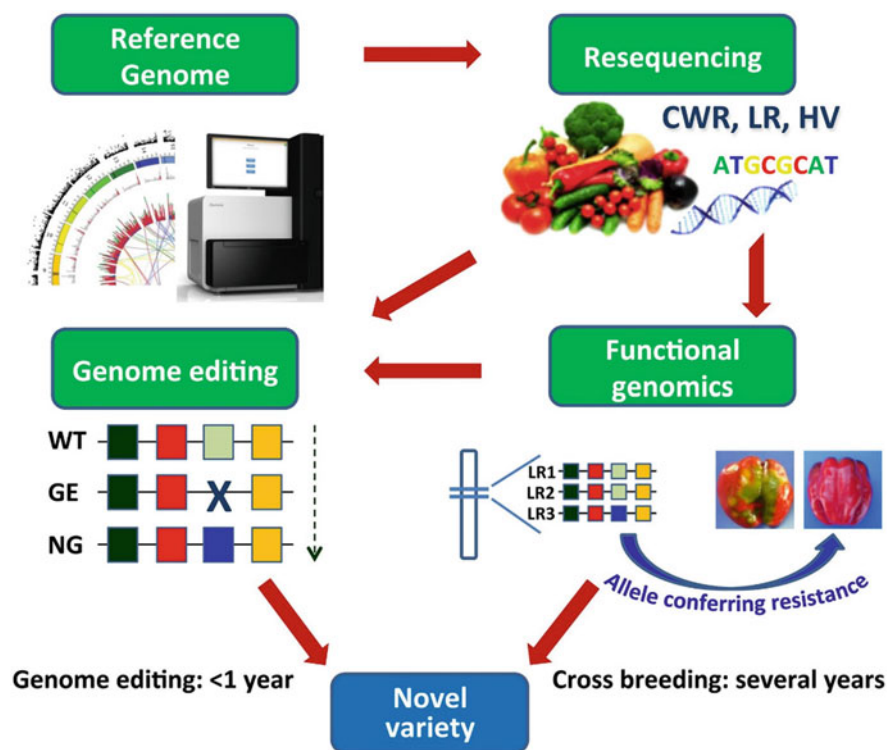


Fig. 8.13 Enhancement and manipulation of genomic resources by cross-breeding and by genome editing. Resequencing of crop wild relatives (CWR), landraces (LR) and heirloom varieties (HV) in vegetables and subsequent detection of the allelic variant associated with relevant attributes permit the introduction of precise genome modifications accelerating the breeding of novel varieties. Wild type (WT), genome editing (GE) process, new genome (NG) edited genotype, landraces (LR 1, 2, 3). Alleles are shown by colored boxes. (Source: Cardi et al. 2017)

8.13 Mutation Breeding in Summer Squash

8.13.1 Conventional Mutagenesis (Seeds)

Artificial mutation induction has proved to be a valuable method to produce variation in many species. In addition, mutation induction techniques may be used to alter a genetic pattern of crop plants, with a strong probability of success. There are few studies on the use of mutagenesis in summer squash or the family Cucurbitaceae to induce mutation. A few numbers of investigations discussed the effect of EMS on germination rate. Most results indicated that the low concentrations did not impact the percentage of germination, although high concentrations lowered it. Among the various mutagenesis gamma rays and ethyl methane sulfate (EMS) were successfully used in several plant species to induce variability in both quantitative and qualitative characteristics (Ahirwar et al. 2014).

A few numbers of studies addressed the impact of gamma irradiation on germination time and percentages. Most results showed that the low doses increased germination percentage and decreased germination period, while the high doses decreased the first trait and increased the second. Several types of chlorophyll mutants were isolated in the M_2 generation after treating seeds of several crops with mutagenesis (Melki and Marouani 2009).

In *C. pepo* Whitwood and Weigle (1978) reported that two compact mutants induced in early prolific Straight Neck with a 0.035 M solution of ethyl methane sulphate (EMS), which segregated for habit in the M_2 , were phenotypically similar to a naturally occurring extreme dwarf of *C. pepo* data obtained from the F_2 of the mutants induced and the extreme dwarf and from their crosses and backcrosses to the F_1 of the normal height crossed with the extreme dwarf, revealed that the mutations induced were operated by a single suppressed gene that controlled the extreme dwarf. Kawaide and Matsuura (1996) exposed seeds and pollen of the monoecious inbred line of cucumber T_1 to gamma-rays and M_2 lines obtained after self-pollination of seed irradiation and non-irradiation-derived M_1 plants. Eleven mutants from seed irradiation and two from pollen irradiation were identified among the M_2 . Segregation in the M_2 indicated that one recessive gene regulated the mutations.

Low doses of gamma rays will stimulate the effect, whereas high doses have an inhibitory effect on the cucumber (Zagorcheva et al. 1985). From another point of view, some significant reports showed that radiation had influenced the number of leaves per plant. The low doses yielded the best results concerning the number of leaves produced, while the high doses reduced this trait. In winter squash *C. maxima* and pumpkin *C. moschata* genotypes, Kurtar et al. (2017) determined the semi-lethal gamma-ray doses for initiation of a genetic variant. The seeds were therefore irradiated at five separate gamma-ray doses (50, 100, 200, 300 and 400 Gy) by a cobalt-60 (^{60}Co) source and the efficacy of irradiation doses was checked for seed and seedling observations. García et al. (2019) examined the phenotypes of single and double-modified homozygous and heterozygous plants showing that the two ethylene receptors help in regulating the ethylene reaction.

8.13.2 Enhanced Traits and Improved Cultivars

In any economic crop, the yield is the main goal. This critical object was improved by various means. The radiation toll has played a major role in this issue. Treatments with low doses of gamma rays were used to stimulate early and total yield, while the higher doses reduced yield and its component. Li et al. (1994) treated *C. moschata* seeds with gamma rays of ^{60}Co . In plants of M_1 generation, there was a marked variation in fruit shape and weight, even within a single plant.

Soltysiak and Kubicki (1988) stated that in cucumber, the EMS-induced line of the cultivar Borszczagowski, characterized by a hypocotyl two-thirds shorter than the normal and reduced length of internode, was crossed with lines of different habit, the color of the fruit spine and type of sex.

The segregation ratio indicated that the mutation expression was dependent upon a recessive gene. In the other study, Jiawang et al. (1997) irradiated inbred cucumber line dry seeds with a gamma rays dose of 90,000 Roentgens, and mutants that had excellent comprehensive traits were developed. The inbred line M_8 , which had many beneficial traits, was produced after three generations of inbreeding and selection. A new F_1 line, tolerant to low temperature and low-intensity light and suitable for greenhouse plastic cultivation, was derived by crossing M_8 with another inbred line. The hybrid was earlier maturity, higher yield and was more disease resistant than its parents.

There was almost no branching, and the morphology of the flower differed significantly from the previous species mutation. Abd El-Rahman (2000) also treated the *Citrullus colocynthis* seeds with gamma rays of 0, 5, 10, 15 and 20 KR. He found that the variations between mutant lines in the M_3 generation were highly considerable for stem length, the number of leaves and branches for each plant and plant dry weight. For at least one of the four character measures, all mutant lines varied significantly from the original parent. Asbah (2007) studied the efficacy of mutagenesis in especially ethyl methane sulphate (EMS), and gamma-rays in the mutation process in two summer squash cultivars, namely Eskandarani as well as causing genetic variation for mutant selection with desirable characteristics. Test the mutagenesis effect of both 10, 20, 30 and 40 Kr gamma irradiation and EMS at 0.025, 0.05, 0.1 and 0.2% concentrations on two summer squash cultivars, Eskandarani and Gabla as an attempt to induce valuable productivity mutations. García et al. (2018) registered 20 randomly distributed chromosomes in squash. The mutations were most influencing intergenic zones, but 7.9 and 6.0% of the mutations were found in L_1 and L_2 .

8.14 Hybridization

8.14.1 Conventional Hybridization

Hybridization is the best way to improve cross-pollinated crops such as summer squash. Several researchers have used squash inbred lines have been used to produce F_1 hybrids and study the nature of the genetics and characteristics of these hybrids. The breeders of summer squash have effectively exploited heterogeneity in yield characters to develop desirable hybrids through the crossing of inbred lines. Increased F_1 hybrid performance has encouraged the seed men to spend many dollars on developing novel F_1 hybrids. The breeders must examine and evaluate a huge number of cultivars and inbred lines in cross groups and evaluate them in different seasons and sites to select those hybrids that have shown high yield, good characteristics and high stability (Muluaem and Abate 2016).

Cucurbit plants are joined to various species of rootstock and assortments using a set of strategies for uniting. The usually unified cucurbit crops include watermelon, melon and cucumber. Cucurbit breeders have always been curious about crosses between species of *Cucurbita*. The collection of the most amazing interspecific cross combinations is crucial to the breeding of vegetable rootstocks. The inter-specific rootstocks are nowadays used widely to join the watermelon, melon and cucumber (Lee et al. 2010). Other strategies, bridge cross counting (Zhang et al. 2012), bud fertilization (Hayase 1961), rehashed fertilization (Yongan et al. 2002) and using development controllers (Nascimento et al. 2007) were used in efforts to advance the victory of interspecific crossing (Lebeda et al. 2007).

The variable evidence shows that selection during the sale is not particularly effective, raising the frequency of desirable genes of small effect, especially genes involved in controlling quantitative inheritance traits (Allard 1971). *C. pepo* exhibits great differences in fruit size, shape and color. Paris proposed a classification of the species comprising eight groups of cultivars based on the fruit shape, a polygenic characteristic. Numerous botanists have described the successful, refined embryos from the production of natural products at *C. pepo* (Kwack and Fujieda 1987; Loy 2012; Sisko et al. 2003). Planted *Cucurbita* species do not have the same inbreeding and heterosis behavior as other cross-pollinated crops such as *Zea mays* and *Allium cepa* (Jansi et al. 2018; Whitaker and Davis 1962).

8.14.2 Heterosis and Hybrid Vigor

Different breeding strategies depend on the selection of suitable hybrids, in addition to the degree of heterosis, which required a unique specific linking power. The breeders often challenge the selection of parents and hybrids (Bocianowski et al. 2015). Breeding for high-yielding crop varieties,

The heterogeneity of the superior parent in the number of fruits per plant and earliness was found within the genetic examination of the summer squash cultivar Eskandarani (Abdein 2005; Metwally et al. 1988). Abdein (2005) studied the

variations in fruits, shapes and the size of certain crosses and their reciprocals from the photographic images (Fig. 8.14).

There are several studies for estimating heterosis on summer squash. Heterosis is considered to be a common occurrence in all plants. Firpo et al. (1998) crossed between 10 selected summer squash inbred lines. They found that for the number and diameter of extended leaves and plant length, most crosses exhibited significant values of heterogeneity compared to normal parents. Obiadalla (2006) recorded heterosis in squash for days until the emergence of the first female flower, and the sex ratio was evaluated as the number of female flowers/number of total flowers. In the summer squash, Al-Hamdany and Al-Lelah (2010) measured four parents and their hybrids to estimate heterogeneity using a 4×4 cross over the entire night.

Marie et al. (2012) studied heterosis concerning the better parent. For the hybrid, IL 3 \times IL 8, negative heterobeltiosis of -13.71% was obtained for the number of nodes to the first flower trait. El-Khatib (2013) observed that the evaluated quantity of heterogeneity in the mid-parents demonstrated positive and negative high significant values, except that some hybrids were negligible for vegetative attributes at both sites and their pooled data. In a 7×7 half-diallel cross mating pattern, Albrifcany (2015) studied heterosis in early traits. More recently, summer squash F_1 hybrids have been used to enhance production and earliness characteristics (Habiba et al. 2015; Hussien 2015; Hussien and Hamed 2015; Karipcin and Inal 2017).

8.14.3 Genetic Parameters and Nature of Gene Action

Summer squash belonging to the species *C. pepo* contains different varieties. These varieties are different in vegetative characters, flowering times, yield and its components traits. Also, the fruits that make up the edible plant component vary significantly in length, thickness, shape and color. Thus, among all collections of various varieties, breeders would find great variations.

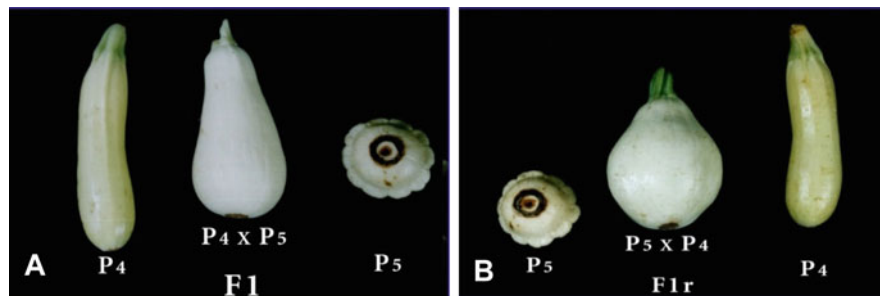


Fig. 8.14 *Cucurbita pepo* fruits from hybridization, (a) F_1 hybrid $P_4^{\ominus} \times P_5^{\ominus}$ and (b) F_{1r} reciprocal hybrid $P_5^{\ominus} \times P_4^{\ominus}$. (Source: Abdein 2005)

A lot of investigation on summer squash has been performed to identify the types of gene action associated with this phenomenon. Hence, studying the degrees of difference and nature of gene behavior among several selected varieties that are being utilized would be very useful. In this area, three major matching systems are used as follows, full diallel, half-diallel and line \times tester matching designs (Bocianowski et al. 2015; El-Shoura and Abed 2018; Patel et al. 2010).

8.14.3.1 Complete Diallel Mating Design

The complete diallel cross has the advantage of generating F_1 and their reciprocal F_1 . The reciprocal hybrids benefit from modifying the parental at the cross by utilizing one parent as a female in one cross and utilizing it as a male parent in its reciprocal cross. So, it would be very important to evaluate and study the differences in F_1 hybrid performance and F_{1r} reciprocal performance for all traits. The obtained differences were positive for their reciprocals from certain crosses and negative for each other since the complete diallel crosses mating design presents estimates of general combining ability (GCA), specific combination ability (SCA) and reciprocal results. Those components will develop into conditions with genetic variation. GCA estimates additive ($\delta^2 A$), while SCA is a non-additive approximation or dominance ($\delta^2 D$) (Abdein 2016). Al-Ballat (2008) reported that the additive component was important for stem length and the number of leaves per plant and highly significant for both traits was the dominance of genetic variations.

Al-Hamdany and Al-Lelah (2010) assessed four parents and their hybrids to assess the genetic variability for vegetative characteristics by utilizing four summer squash varieties with complete diallel crosses. Douglas et al. (2011) confirmed the effects of an additive and non-additive gene on parthenocarpy expression and papaya ringspot virus (PRSV-W) resistance were very important. Sanin et al. (2014) examined the predominance of additive gene action on the dominant type for the early traits.

El-Shoura and Abed (2018) studied heterosis and the combined ability for squash hybrid development. GCA was important for all characteristics except early yield per plant and shape index. SCA was significant for all characteristics except for the number of female flower nodes and the initial yield of the plant. The determined mean GCA/SCA squares showed that the effects of the additive gene, except for the first yield per plant and vitamin C, were the main influence on the inheritance of all traits studied. GCA estimates were also larger for most of the traits studied than their respective SCA estimates.

8.14.3.2 Half Diallel Mating Design

The half diallel analysis is a type of mating system that assists and enables the breeder to obtain estimates of the GCA and SCA. These estimates are indicators of additive and non-additive genetic differences, respectively. The half-diallel mating design may be used for GCA and SCA estimation. Thus, diallel crosses are sometimes used in genetic studies to assess the inheritance nature (additive and non-additive) of the traits examined (Abdein et al. 2017; Al-Araby 2010; Hussien 2015; Obiadalla 2006). Abou El-Nasr et al. (2010) observed that, for all characters

studied, the proportion between the mean squares of GCA and SCA showed that the additive component of genetic variation played a major role in heredity. Abd El-Hadi et al. (2013) showed in the summer squash that the parental variety Zucchini Nova Verde di Milano was the best compound for the number of leaves per plant.

Albrifcany (2015) used seven squash varieties in a half-diallel cross-mating design and observed yield traits. Hussien and Hamed (2015) found that GCA magnitude was always greater than SCA and the GCA/SCA ratios were higher than unity. They accept the significance of additive and non-additive genetic variation in the inheritance in summer squash hybrids of important traits (El-Shoura and Abed 2018).

8.14.3.3 Factorial (Line \times Tester) Mating Design

Sprague and Tatum (1942) utilized the terms GCA and SCA to the average performance of the parental line and hybrid combination. Ahmed et al. (2003) used the summer squash line \times tester procedure to evaluate the combined power magnitudes by measuring the components of factorial mating designs. Analysis of line \times testers provides information about GCA and SCA effects of combining ability, and this would help estimate different types of genetic variation actions. The x-line tester analysis method is also used to assess beneficial parents and hybrids (Bocianowski et al. 2015).

Ghai et al. (1998) measured a factorial mating design of 10 lines \times 4 testers and their 40 top crosses during the summer squash. El-Sharkawy (2000) researched the combining capability of seven inbred lines obtained from the Eskandarani genotype. She utilized three testers and eight parental inbred lines to regenerate 24 F_1 hybrids. The consequences of σ_{sca}^2 of hybrids, which indicate the non-additive genetic variation, were observed to be greater than those of the other two components in most vegetative traits, i.e. σ_{gca}^2 of lines and σ_{gca}^2 of testers.

Ahmed et al. (2003) assessed combining ability in 7 lines \times 2 tester crosses in a factor layout of summer squash cultivars. He utilized eight inbred summer squash lines from Al-Jebory (2006 and 2008) to approximate genetic parameters of earliness traits. He also utilized eight inbred lines of summer squash and crossed them with six lines as female parents and two lines as male parents and their 12 F_1 hybrids.

Tamilselvi et al. (2015) used line \times tester mating design to analyze combining abilities for earliness in squash. On squash, Davoodi et al. (2016) examined standard heterosis in vegetative and qualitative traits in *C. pepo* and *C. moschata* fruit-type hybrids. Recently, Al-Araby et al. (2019) studied the heterosis and combining ability in cucumber (*Cucumis sativus* L.) using line \times tester analysis.

8.14.3.4 Homogeneity Test

Abdein (2016) studied the homogeneity test for the mean square from factorial breeding design and complete diallel crosses mating design. The results of the homogeneity test revealed that the mean squares of GCA from the complete diallel crosses mating design were more effective and bigger in amounts than their elements of mean squares from the factorial mating design for males and females. These

results showed that additional genetic variation appears to be significant when evaluated by utilizing male or female components. Instead, the predominant genetic variance appears to be crucial when assessed from a factor mating design, since the mean square of the male by the female was greater than the mean square of SCA. Generally, in light of this analysis, calculating the additional genetic variance will be more reliable than the nature of factorial mating, and the opposite for dominance genetic variance that will be calculated to use the diallel crosses more precisely. Consequently, all patterns of mating can be used to predict genetic variances with a variable degree of efficiency.

8.15 Conclusions and Prospects

Many efforts should be spent on genetic conservation of different landraces and developing cultivars resistant to the pathogen, especially *Cucurbita* is one of the most essential crops in a developing country. A gene bank in each country should cooperate with the ministry of agriculture in developing and evaluating the new genetic engineering tools in increasing yield productivity. Karyotype, in addition to genetic map to produce quantitative trait loci (QTL) should be utilized for the advancement of qualitative and quantitative characteristics. The report concludes that people as of now utilize about half of the plant arrive for nourishment generation and, as worldwide populace levels rise, agricultural arrive is reaching to be in exceptionally brief supply. Typically, one or more of the impacts of climate change will be a decline in agrarian efficiency over the tropics, meaning that we are going to have to cut down woodlands and change over unused arrive into farmland. This deforestation will lead to indeed more carbon emanations, coming full circle in a horrendous cycle of expanding warming.

References

- Abd El-Al ZE, Khalaf-Allah MA (1973) Effect of visual selection and inbreeding on some quantitative characters of summer squash. *Alex J Agric Res* 21(2):277–282
- Abd El-Hadi AH, Zaghoul MM, Gabr AH (2004) Nature of gene action, heterosis and inbreeding depression of yield and yield component traits in squash (*Cucurbita pepo* L.). *Zag. J Agric Res* 31(6):2707–2725
- Abd El-Hadi AH, El-Adl A, Hamada M et al (2005) Manifestation of heterosis and genetic parameters associated with it for some vegetative and earliness traits in squash. *J Agric Sci Mans Univ* 30(3):1363–1379
- Abd El-Hadi AH, Farid SM, El-Khatib EH (2013) Combining ability and genetic variance components of a diallel crosses among some squash varieties. *J Agric Sci Mans Univ* 4(3): 119–131
- Abd El-Hadi AH, El-Adl AM, Fathy HM et al (2014) Heterosis and genetic behavior of some yield and yield component traits in squash (*Cucurbita pepo* L.). *Alex Sci Exc J* 35(3):178–189. <https://doi.org/10.21608/asejaiqjsae.2014.2609>
- Abd El-Maksoud MM (1986) Nature of gene action of economic traits in squash (*Cucurbita pepo* L.). M. Sc. Thesis, Faculty of Agriculture, Mansoura University, Egypt

- Abd El-Maksoud MM, El-Adl AM, Hamada MS et al (2003) Inheritance of some economical traits in squash (*Cucurbita pepo* L.). J Agric Sci Mans Univ 28(6):4463–4474
- Abd El-Rahman MM (2000) Inducing genetic variability in *Citrullus colocynthis* by using gamma irradiation. J Agric Sci Mans Univ 25(1):193–199
- Abd El-Salam MM, El-Demardash IS, Hussein AH (2010) Phenotypic stability analysis, heritability and protein patterns of snake cucumber genotypes. J Amer Sci 6(12):503–507
- Abdein MA (2005) Quantitative genetics of some economic traits in squash (*Cucurbita pepo* L.). Thesis, Faculty of Agriculture, Mansoura University, Egypt, M. Sc
- Abdein MA (2016) The performance of parental lines and their hybrids resulted from diallel crosses mating design in squash (*Cucurbita pepo* L.). Ph. D. Thesis, Faculty of Agriculture, Mansoura University, Egypt
- Abdein MA (2018) Genetic diversity between pumpkin accessions growing in the Northern Border Region in Saudi Arabia based on biochemical and molecular parameters. Egypt J Bot 58(3): 463–476. <https://doi.org/10.21608/ejbo.2018.3612.1171>
- Abdein MA, Fathy HM, Hikal DM (2017) General performance, combining abilities, and heritability of yield and yield component traits in pumpkin (*Cucurbita moschata* Poir.) at different conditions. KMITL-STJ 17(1):121–129
- Abdel Sayyed SM, Mahgoub SM, Emam YT et al (2003) Genetically studies on sweet melon fruit sensory quality characters. Zaga J Agric Res 30(4):1553–1564. https://doi.org/10.1007/7397_2016_29
- Abdel-Rahman KM (2006) Effect of pumpkin seed (*Cucurbita pepo* L.) diets on benign prostatic hyperplasia (BPH): chemical and morphometric evaluation in rats. World J Chem 1(1):33–40
- Abou El-Nasr ME, Tolba MH, Ahmed HM (2010) Combining ability for some characters in summer squash (*Cucurbita pepo* L.). J Plant Prod Mans Univ 1(5):663–671
- Acosta-Patino JL, Jimenez-Balderas E, Juarez-Oropeza MA et al (2001) Hypoglycemic action of *Cucurbita ficifolia* on type 2 diabetic patients with moderately high blood glucose levels. J Ethnopharmacol 77(1):99–101. [https://doi.org/10.1016/s0378-8741\(01\)00272-0](https://doi.org/10.1016/s0378-8741(01)00272-0)
- Ahirwar RN, Lal JP, Singh P et al (2014) Gamma-rays and ethyl methane sulphonate induced mutation in microsperma lentil (*Lens culinaris* L. Medikus). SGPB 9(2):791–795
- Ahmed EA, Ibn-Oaf HS, El Jack AE (2003) Combining ability and heterosis in line × tester crosses of summer squash (*Cucurbita pepo* L.). Cucurbit Genet Coop Rep 26:54–56
- Ajuru M, Nmom F (2017) A review on the economic uses of species of Cucurbitaceae and their sustainability in Nigeria. Amer J Plant biol 2(1):17–24. <https://doi.org/10.11648/j.ajpb.20170201.14>
- Al-Araby AA (2010) Estimation of heterosis, combining ability and heritability in inter varietals crosses of summer squash (*Cucurbita pepo* L.). Ph.D. Thesis, Faculty of Agriculture, Tanta University, Egypt
- Al-Araby ME, Ahmed SA, Omran et al (2019) Heterosis and combining ability in cucumber (*Cucumis sativus* L.) using line × tester analysis. Egypt J Plant Breed 23(6):1169–1194
- Al-Ballat IA (2008) Breeding studies on summer squash crop (*Cucurbita pepo* L.). M. Sc. Thesis, Faculty of Agriculture, Tanta University, Egypt
- Albrifcany MT (2015) Estimation of heterosis and nature of gene action for some economical traits in squash (*Cucurbita pepo* L.). M. Sc. Thesis, Faculty of Agriculture Mansoura University, Egypt
- Al-Hamdany SY, Al-Lelah HWB (2010) Estimating of heterosis and genetic variability in summer squash (*Cucurbita pepo* L.). J Rafedin Agric Mosul Iraq 38(4):316–327. <https://doi.org/10.33899/magrj.2010.28007>
- Al-Jebory KDH (2006) Heterosis and genotypic, phenotypic and environmental correlations for several characters of summer squash. Iraqi J Agric Sci 37(3):45–58
- Al-Jebory KDH (2008) Estimation of some genetic parameters and heterosis for summer squash growth, yield and its NPK content. J Alanbar Agric Sci 6(1):146–157
- Allard RW (1971) Principles of plant breeding. Edgard Blüchner, São Paulo, p 381

- Al-Tamimi J, Attyaf T (2014) Genetic fingerprint of some *Cucurbita pepo* (summer squash) genotypes using molecular and biochemical techniques. *Magazin Al-Kufa Univ Bio* 6(1): 2073–2086
- Ananthkrishnan G, Xia X, Elman C et al (2003) Shoot production in squash (*Cucurbita pepo*) by in Vitro organogenesis. *Plant Cell Rep* 21:739–746
- Araujo EF, Mantovani EC, Da Silva RF (1982) Influence of fruit age and storage period on squash seed quality. *Rev Brasil de Semen* 4:77–87. <https://doi.org/10.9734/JEAI/2017/36296>
- Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. *Plant Mol Biol Rep* 9(3):208–218
- Asbah AM (2007) Studies on breeding and improvement of summer squash (*Cucurbita pepo* L.) through mutagenesis and selection. Ph.D. Faculty of Agriculture, Ain Shams University, Egypt
- Badawi MA, Metwally E, Taha SS et al (2008) Large scale production of haploid plants by un-pollinated ovules culture in squash (*Cucurbita pepo* L.). *J Agric Sci Mans Univ* 33(7): 4981–4992
- Bahlgerdi M (2014) The study of plant density and planting methods on some growth characteristics, seed and oil yield of medicinal pumpkin (*Cucurbita pepo* Var. *Styriaca*, Cv. *Kaki*). *Am J Life Sci* 2:319–324. <https://doi.org/10.11648/j.ajls.20140205.21>
- Bailey LH (1929) The domesticated cucurbits. *Genet Herb* 2:23–34
- Balkaya A, Kandemir D (2015) An overview of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) growing in Turkey. *Azarian J Agric* 2(3):57–64
- Balkaya A, Yildiz S, Horuz A et al (2016) Effects of salt stress on vegetative growth parameters and ion accumulations in Cucurbit rootstock genotypes. *J Plant Breed Genet* 2(2):11–24
- Baranek M, Stift G, Vollmann J et al (2000) Genetic diversity within and between the species *Cucurbita pepo*, *C. moschata* and *C. maxima* as revealed by RAPD markers. *Rep Cucurbit Genet Coop* 23:73–77
- Blaylock AD (1994) Soil salinity, salt tolerance and growth potential of horticultural and landscape plants. Cooperative Extension Service, University of Wyoming, Circular B-988, p 300
- Bocianowski J, Nowosad K, Brzeskwiniewicz H et al (2015) Finding ranking of testers in line × tester experiments. *Amer J Current Genetics* 1:1–9
- Bolotin A, Quinquis B, Sorokin A, Ehrlich SD (2005) Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology* 151: 2551–2561
- Borghi B, Magglore T, Boggini et al (1973) Inbreeding depression and heterosis in *Cucurbita pepo* L. evaluated by means of diallel analysis. *Genetical Agrouria* 27(4) 415–432
- Bowley SR, Taylor NL (1987) Introgressive hybridization. CRC handbook of plant science in agriculture, vol 1. CRC Press, Boca Raton, FL
- Brewbaker JL (1964) Agricultural genetics. Prentice-Hall, Inc, Englewood Cliffs, NJ. <https://doi.org/10.1002/bimj.19670090214>
- Brown RN, Myers JR (2002) A genetic map of squash (*Cucurbita* spp.) with randomly amplified polymorphic DNA markers and morphological markers. *J Am Soc Hortic Sci* 127:568–575
- Brush SB (1995) In Situ Conservation of landraces in centers of crop diversity. *Crop Sci* 35:346–354
- Byrne PF, Volk GM, Gardner C et al (2018) Sustaining the future of plant breeding: the critical role of the USDA-ARS national plant germplasm system. *Crop Sci* 58(2):451–468
- Caili FU, Huan SHI, Quanhong LI (2006) A review on pharmacological activities and utilization technologies of pumpkin. *Plant Food Hum Nutr* 61(2):73–80. <https://doi.org/10.1007/s11130-006-0016-6>
- Cardi T, D’Agostino N, Tripodi P (2017) Genetic transformation and genomic resources for next-generation precise genome engineering in vegetable crops. *Front Plant Sci* 8:241. <https://doi.org/10.3389/fpls.2017.00241>
- Carlquist S (1992) Wood anatomy of selected Cucurbitaceae and its relationship to habit and systematics. *Nord J Bot* 12:347–355. <https://doi.org/10.1111/j.1756-1051.1992.tb01312.x>

- Carol G, Baodi X, Dennis G (1995) Somatic embryogenesis and regeneration from cotyledon explants of six squash cultivars. *HortSci* 30:1295–1297
- Carvajal F, Palma F, Jiménez-Muñoz R et al (2018) Changes in the biosynthesis of cuticular waxes during postharvest cold storage of zucchini fruit (*Cucurbita pepo* L.). *Acta Horti* 1194:1475–1480. <https://doi.org/10.17660/ActaHort.2018.1194.206>
- Chaney L, Sharp AR, Evans CR et al (2016) Genome mapping in plant comparative genomics. *Trends Plant Sci* 21:770–780
- Chee P (1991) Somatic embryogenesis and plant regeneration of squash *Cucurbita pepo* L cv. YC 60. *Plant Cell Rep* 9:620–622. <https://doi.org/10.1007/BF00231801>
- Chee P (1992) Initiation and maturation of somatic embryos of squash (*Cucurbita pepo*). *HortSci* 27:59–60. <https://doi.org/10.21273/HORTSCI.27.1.59>
- Choudhary H, Ram HH (2000) Characterization of indigenous muskmelon germplasm lines based on SDS-PAGE of seed protein. *Veg Sci* 27(1):35–38
- Clark RL, Widrechner MP, Reitsma KR, Block CC (1991) *Cucurbit* germplasm at the north central regional plant introduction station, Ames. *Iowa HortSci* 26(4):326–451
- Clough GH, Hamm PB (1995) Coat protein transgenic resistance to watermelon mosaic and zucchini yellow mosaic virus in squash and cantaloupe. *Plant Dis* 79:1107–1109
- Collinge DB, Jørgensen HJ, Lund OS et al (2010) Engineering pathogen resistance in crop plants: current trends and future prospects. *Annu Rev Phytopathol* 48:269–291
- Cruz-Cruz CA, González-Arnao MT, Engelman F (2013) Biotechnology and conservation of plant biodiversity. *Reso* 2(2):73–95. <https://doi.org/10.3390/resources2020073>
- Cuevas-Marrero H, Wessel L (2008) Morphological and RAPD marker evidence of gene flow in open pollinated populations of *Cucurbita moschata* interplant with *C. argyrosperma*. In: Pitrat M (ed) *Cucurbitaceae, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*. 21–24th May, 2008, INRA, Avignon (France), pp 347–352
- Curtis LC (1940) Heterosis in summer squash (*Cucurbita pepo*) and the possibilities of producing F1 hybrid seed for commercial planting. *Proc Amer Soc Hort Sci* 37:827–828. <https://doi.org/10.13140/RG.2.2.18468.94088>
- Dahiya MS, Pandita ML, Vashistha RN (1990) Studies on variability and heritability in summer squash (*Cucurbita pepo* L.). *Res Develop Rep* 7:102–105
- Dalda-Şekerci A, Karaman K, Yetişir H (2020) Characterization of ornamental pumpkin (*Cucurbita pepo* L. var. *ovifera* (L.) Alef.) genotypes: molecular, morphological and nutritional properties. *Genet Resour Crop Evol* 67:533–547. <https://doi.org/10.1007/s10722-020-00883-x>
- Danida (2002) Assessment of potentials and constraints for development and use of plant biotechnology in relation to plant breeding and crop production in developing countries. Ministry of Foreign Affairs, Denmark
- Darrudi R, Nazeri V, Soltani F et al (2018) Evaluation of combining ability in *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne accessions for fruit and seed quantitative traits. *J Appl Res Med Aromat Plants* 9:70–77. <https://doi.org/10.1016/j.jarmap.2018.02.006>
- Davoodi S, Olfati JA, Hamidoghli Y et al (2016) Standard heterosis in *Cucurbita moschata* and *Cucurbita pepo* interspecific hybrids. *Int J Veg Sci* 22(4):383–388. <https://doi.org/10.1080/19315260.2015.1042993>
- Debeaujon I, Branchard M (1993) Somatic embryogenesis in Cucurbitaceae. *Plant Cell Tissue Organ Cult* 34:91–100. <https://doi.org/10.1007/BF00048468>
- Decker DS (1988) Origin(s), evolution, and systematics of *Cucurbita pepo* (Cucurbitaceae). *Econ Bot* 42:4–15. <https://doi.org/10.1007/BF02859022>
- Decker-Walters DS, Walters TW, Cowan CW et al (1999) Isozymic characterization of wild populations of wild populations of *Cucurbita pepo*. *J Ethnobiol* 13:55–22
- Del Valle Echevarria AR, Campbel A, Radovich TJ et al (2020) Quantitative trait loci (QTL) analysis of fruit and agronomic traits of tropical pumpkin (*Cucurbita moschata*) in an organic production system. *Horticultrae* 6(1):14
- Diez MJ, Pico B, Nuez F (2002) Compilers (2002) *Cucurbit* genetic resources in Europe. Ad hoc meeting, 19 January 2002, IBPGR, Rome

- Domblides EA, Kan LY, Khimich GA et al (2018) Cytological Assessment of doubled haploids in summer squash (*Cucurbita pepo* L.). *Vegetable Crop Russia* 6:3–7. <https://doi.org/10.18619/2072-9146-2018-6-3-7>
- Douglas WN, Maluf WR, Figueira AR et al (2011) Combining ability of summer-squash lines with different degrees of parthenocarpy and PRSV-W resistance. *Genet Mol Biol* 34(4):616–623. <https://doi.org/10.1590/S1415-47572011005000039>
- El-Adl AM, Abd El-Hadi AH, Fathy HM et al (2012) Molecular genetic evaluation of seven varieties of summer squash. *J Am Sci* 8(5):41–48
- El-Diasty ZM, Kash KS (1989) The importance of additive and non-additive genetic variances estimated from diallel and factorial mating designs in squash (*Cucurbita pepo* L.). II. Inbreeding depression and types of gene action associated with it. *J Agric Sci Mans Univ* 14(1):233–242
- El-Gazar TM (1981) Combining ability and manifestation of heterosis in squash (*Cucurbita pepo* L.). Ph. D. Thesis, Faculty of Agriculture, Mansoura University, Egypt
- El-Gazar TM, Zaghoul MM (1983) Inheritance of some quantitative traits in squash crosses (*Cucurbita pepo* L.). *J Agric Sci* 8(2):358–372
- El-Gendy SA (1999) Estimation of genetic parameters in some squash hybrids through two mating designs. Ph. D. Thesis, Faculty of Agriculture Mansoura University, Egypt
- Elinge CM, Muhammad A, Atiku FA et al (2012) Proximate, mineral and anti-nutrient composition of pumpkin (*Cucurbita pepo* L.) seeds extract. *Int. J Plant Res* 2(5):146–150. <https://doi.org/10.5923/j.plant.20120205.02>
- El-Khatib EH (2013) Genetic behavior of some economical traits in squash (*Cucurbita pepo* L.). M. Sc. Thesis, Faculty of Agriculture, Mansoura University, Egypt
- El-Shamy MM (2009) Molecular analysis of cucumber mosaic cucumovirus symptoms development on squash plants. *J Appl Sci* 6(8):94–103
- El-Sharkawy GAM (2000) An analytical study for the genetic behavior of some important characters of summer squash (*Cucurbita pepo* L.) using a diallel cross system among seven inbred lines of Eskandarani cultivar. M. Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt
- El-Shawaf LL, Abd-Alla SA, El-Aidy F et al (1986) Inheritance of yield and related traits in summer squash (*Cucurbita pepo*). *Ann Agric Sci Moshtohor* 24(2):911–928
- El-Shoura AM, Abed MY (2018) Heterosis and combining ability for development of squash hybrids (*Cucurbita pepo* L.). *J Plant Prod* 9(12):1181–1187
- El-Tahawy M (2007) Genetically studies on the most important economical characteristics of some squash cultivars in Egypt. M. Sc. Thesis, Faculty of Agriculture, Suez Canal Univ, Egypt
- Engelmann F (2004) Genetic resource conservation of seeds. *Encyclopedia of Plant and Crop Science*. Marcel Dekker Inc, New York
- Esteras C, Nuez F, Picó B (2011) Genetic diversity studies in Cucurbits using molecular tools. In: Wang Y, Behera TK (eds) *Cucurbits: genetics, genomics and breeding in crop plants*. Science Publishers, Enfield, New Hampshire., pp 25. <https://doi.org/10.1201/b11436-6>
- Esteras C, Gómez P, Monforte AJ et al (2012) High-throughput SNP genotyping in *Cucurbita pepo* for map construction and quantitative trait loci mapping. *BMC Genomics* 13(1):80
- Fang EF, Yi CZ, Bun T et al (2011) Momordica charantialelectin, a type II ribosome inactivating protein, exhibits antitumor activity toward human nasopharyngeal carcinoma cells *in vitro* and *in vivo*. *Cancer Prevent Res* 5(1):109–121
- FAO (1996) Report on the state of the world's plant genetic resources. Food and Agricultural Organization of the United Nation, Rome, Italy
- FAO (2018) FAOSTAT Agricultural Database. Food and Agriculture Organization of the United Nations, Rome, Italy <http://www.fao.org/faostat/en/#data/QC/visualize>)
- Fayeun LS, Odiy AC, Makinde SC et al (2012) Genetic variability and correlation studies in the fluted pumpkin (*Telfairia occidentalis* Hook F.). *J Plant Breed Crop Sci* 4(10):156–160. <https://doi.org/10.5897/JPBCS12.011>
- Fehr RW (1987) Principles of cultivar development, vol 2. Macmillan Pub Co, New York

- Ferriol M, Pico B (2008) Pumpkin and winter squash. In: Prohens J, Nuez F (eds) HDB plant breeding. Springer, Heidelberg, pp 317–349
- Ferriol M, Picó B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor Appl Genet* 107:271–282. <https://doi.org/10.1007/s00122-003-1242-z>
- Firpo II, Anido FL, Gareia SM et al (1998) Heterosis in summer squash (*Cucurbita pepo* L.). *Cucurbit Genet Coop Rep* 21:43–45
- Formisano GC, Roig C, Esteras MR et al (2012) Genetic diversity of Spanish *Cucurbita pepo* landraces: an unexploited resource for summer squash breeding. *Genet Resour Crop Evol* 59: 1169–1184. <https://doi.org/10.1007/s10722-011-9753-y>
- Fuchs M, Gonsalves D (2007) Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies. *Annu Rev Phytopathol* 45:173–202
- Fuchs M, Tricoli DM, Carney KJ et al (1998) Comparative virus resistance and fruit yield of transgenic squash with single and multiple coat protein genes. *Plant Dis* 82:1350–1356
- Gaba V, Zelcer A, Gal-On A (2004) Cucurbit biotechnology-the importance of virus resistance. *Vitro Cell Dev Biol Plant* 40(4):346–358
- Gabr AH (2003) Nature of gene action and performance of hybrids in squash (*Cucurbita pepo*, L.). M. Sc. Thesis, Faculty of Agriculture, Mansoura University, Egypt
- García A, Aguado E, Genis P et al (2018) Phenomic and genomic characterization of a mutant platform in *Cucurbita pepo*. *Front Plant Sci* 9(1049):1–13. <https://doi.org/10.3389/fpls.2018.01049>
- García A, Aguado E, Martínez C et al (2019) The ethylene receptors *CpETRIA* and *CpETR2B* cooperate in the control of sex determination in *Cucurbita pepo*. *J Exp Bot* 71(1):154–167. <https://doi.org/10.1093/jxb/erz417>
- Gay C, Kerhoas C, Dumas C (1987) Quality of stress-sensitive *Cucurbita pepo* pollen. *Planta* 171: 82–87. <https://doi.org/10.1007/BF00395070>
- Ghai TR, Jaswinder S, Arora SK et al (1998) Heterosis studies for earliness and yield in summer squash (*Cucurbita pepo* L.). *Punjab Veg Grower* (33):35–40
- Gill NS, Bali M (2012) Evaluation of antioxidant, antiulcer activity of 9- β -methyl-19-norlanosta-5-ene type glycosides from *Cucumis sativus* seeds. *Res J Med Plant* 6:309–317. <https://doi.org/10.3923/rjmp.2012.309.317>
- Glew RH, Glew R, Chuang L et al (2006) Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp.) and *Cyperus esculentus* nuts in the Republic of Niger. *Plant Foods Hum Nutr* 61(2):51–56. <https://doi.org/10.1007/s11130-006-0010-z>
- Gong L, Stift G, Kofler R et al (2008) Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. *Theor Appl Genet* 117:37–48. <https://doi.org/10.1007/s00122-008-0750-2>
- Gong L, Paris HS, Nee MH et al (2012) Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. *Theor Appl Genet* 124:875–891. <https://doi.org/10.1007/s00122-011-1752-z>
- Gong L, Paris HS, Stift G et al (2013) Genetic relationships and evolution in *Cucurbita* as viewed with simple sequence repeat polymorphisms: the centrality of *C. okeechobeensis*. *Genet Resour Crop Evol* 60:1531–1546. <https://doi.org/10.1007/s10722-012-9940-5>
- Gonsalves C, Xue B, Gonsalves D (1995) Somatic embryogenesis and regeneration from cotyledon explants and six squash cultivars. *HortSci* 30:1295–1297. <https://doi.org/10.21273/hortsci.30.6.1295>
- Gwanama C, Labuschagne MT, Botha AM (2000) Analysis of genetic variation in *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. *Euphytica* 113:19–24. <https://doi.org/10.1023/A:1003936019095>
- Habiba RMM, El-Adl AMM, Othman IAH (2015) Intra and inter-specific hybrids in summer squash. *J Agric Chem Biotech* 6(12):597–613

- Hammer K, Teklu Y (2008) Plant genetic resources: selected issues from genetic erosion to genetic engineering. *J Agric Rural Dev Trop Subtrop* 109:15–50
- Harris-Valle C, Esqueda M, Valenzuela–Soto E et al (2011) Tolerance to drought and salinity by *Cucurbita pepo* var. *pepo* associated with arbuscular mycorrhizal fungi of the Sonoran Desert. *Agrociencia* 45(8):959–970
- Hassanein SH, Heakel MY, Abd El-Sayyed S (1975) Heterosis and maternal effects in crosses between inbred lines of summer squash. (*Cucurbita pepo* L.). *Zaga J Agric Res* 2(1):201–213
- Havey M, McCreight J, Rhodes B et al (1998) Differential transmission of the Cucumis organellar genomes. *Theor Appl Genet* 97:122–128. <https://doi.org/10.1007/s001220050875>
- Hayase H (1961) *Cucurbita*-crosses, XIII. Utilization of bud pollination in obtaining interspecific hybrids of *C. pepo* × *C. maxima*. *Jpn J Genet* 25:181–190. <https://doi.org/10.1270/jsbbs1951.11.277>
- Hayes CN, Winsor JA, Stephenson AG (2005) Environmental variation influences the magnitude of inbreeding depression in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *J Evol Biol* 18(1): 147–155
- Heikal A, Hadia HS, Abdel-Razzak et al (2008) Assessment of genetic relationships among and within *cucurbita* species using RAPD and ISSR markers. *J Appl Sci Res* 4(5):515–525
- Herklots GAC (1972) *Vegetables in South-East Asia*. George Allen & Unwin LTD, London
- Hikal DM, Abdein MA (2018) Nutritional and genetically studies on some squash varieties. *Res* 10(12):112–118. <https://doi.org/10.7537/marsrsj101218.14>
- Hussien AH (2015) Nature of gene action and heterotic performance for yield and yield components in summer squash (*Cucurbita pepo* L.). *J Plant Prod* 6(1):29–40. <https://doi.org/10.21608/jpp.2015.49274>
- Hussien AH, Hamed AA (2015) Diallel analysis for studying heterosis and combining ability of some economical yield traits in pumpkin. *J Plant Prod* 6(3):261–270. <https://doi.org/10.3329/agric.v14i1.29097>
- Hutchins AE, Croston FE (1941) Productivity of F₁ hybrids in the squash (*Cucurbita maxima*). *Amer Soc Hort Sci Proc* 39:332–336
- Ishino Y, Shinagawa H, Makino K et al (1987) Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli* and identification of the gene product. *J Bacteriol* 169:5429–5433
- Itah MS, Kwon-Ndung EH (2019) Biometrical evaluation of morphological traits in Family Cucurbitaceae in Lafia, Nigeria. *J Agric Ecol Res Inter* 19(2):1–9. <https://doi.org/10.9734/JAERI/2019/v19i230078>
- Jang TH, Park SC, Yang JH et al (2017) Cryopreservation and its clinical applications. *Integr Med Res* 6(1):12–18
- Jansi V, Rajasree V, Kumar R et al (2018) Heterosis and inbreeding depression studies in pumpkin (*Cucurbita moschata* Duch. ex Poir.). *Electron J Plant Breed* 9(3):1031–1037. <https://doi.org/10.5958/0975-928X.2018.00128.X>
- Jeffrey D (1990) Appendix: an outline classification of the Cucurbitaceae. In: BATES DM, Robinson RW, Jeffrey C (eds) *Biology and utilization of the Cucurbitaceae*. Cornell University, Ithaca and London, pp 449–463
- Jelaska S (1972) Embryoid formation by fragments of cotyledons and hypocotyls in *Cucurbita pepo*. *Planta (Berl)* 103(3):278–280. <https://doi.org/10.1007/BF00386851>
- Jelaska S (1974) Rhizogenesis of pumpkin hypocotyl explants. *Physiol Plant* 31:257–261
- Jiawang L, Zhongkui S, Sen Y et al (1997) A preliminary report on the application of 60 °C gamma-rays to cucumber mutation breeding. *Chin Veg* 2:22–24
- Jinek M, Chylinski K, Fonfara I et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816–821
- Kalaiselvi K, Selvi S (2016) Phytochemical screening and antioxidant activity of *Cucurbita Pepo* leaves. *Eur J Pharm Med Res* 3(6):375–377

- Karipçin MZ, İnal B (2017) Determination of heterosis and heterobeltiosis values of salt-tolerant summer squash (*Cucurbita pepo* L.) genotypes and genetic relationships of parental genomes. *Appl Ecol Env Res* 15(4):779–796. https://doi.org/10.15666/aeer/1504_779796
- Karlova K (2008) Cucurbitaceae genetic resources in the Czech gene bank, current status of the collection. In: Pitrat M (ed) Cucurbitaceae. INRA, Avignon, pp 281–283
- Kartha KK (1981) Meristem culture and cryopreservation-methods and applications. In: Thorpe TA (ed) Plant tissue culture, methods and applications in agriculture. Academic Press, New York, pp 181–212
- Kartha KK, Engelmann F (1994) Cryopreservation and germplasm storage. In: Vasil IK, Thorpe TA (eds) Plant cell and tissue culture. Springer, Dordrecht
- Kathiravan K, Vengedesan G, Singer S, Steinitz B, Paris HS, Gaba V (2006) Adventitious regeneration in vitro occurs a wide spectrum of squash (*Cucurbita pepo*) genotypes. *Plant Cell Tissue Organ Cult* 85:285–295
- Katzir N, Leshzeshen E, Tzuri G et al (1998) Relationships among accessions of *Cucurbita pepo* based on ISSR analysis. In: McCreight JD (ed) Cucurbitaceae 98, ASHS Press, pp 331–335
- Katzir N, Tadmor Y, Tzuri G et al (2000) Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. *Proc Cucurbitaceae Acta Hortic* 510:433–439. <https://doi.org/10.17660/ActaHortic.2000.510.69>
- Kaviani B (2011) Conservation of plant genetic resources by cryopreservation. *Aust J Crop Sci* 5(6):778–800
- Kawaide O, Matsuura S (1996) Cucumber (*Cucumis sativus* L.) mutants segregating in M₂-generation after gamma-ray seed and pollen irradiation. *Cucurbit Genet Coop Rep* 19:4–6
- Kaźmińska K, Hallmann E, Korzeniewska A et al (2020) Identification of fruit-associated QTLs in winter squash (*Cucurbita maxima* Duchesne) using recombinant inbred lines. *Genes* 11(4):419
- Khalil MA, Hassan AH (2013) Genetic Analysis in some Cucurbitaceae plants. *Egypt J Genet Cytol* 42:345–364. <https://doi.org/10.21608/ejgc.2013.9975>
- Kintzios S, Sereti E, Bluchos P et al (2002) Growth regulator pretreatment improves somatic embryogenesis from leaves of squash (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.). *Plant Cell Rep* 21:1–8. <https://doi.org/10.1007/s00299-002-0448-x>
- Kochhar SL (1981) Tropical crops. A textbook of economic botany. Macmillan Press, London, pp 322–328
- Kolawole OT, Abiona FE, Kolawole SO et al (2011) Effect of *Momordica charantia* fruit extract on normal and alloxan diabetic rats. *Inter J Pharmacol* 7(4):532–535. <https://doi.org/10.3923/ijp.2011.532.535>
- Koxyxob (1925) Chromosome number of three Bulgarian Plants. *CR Acad Bulg Sci* 17:491–494
- Krsnik-Rasol M, Jelaska S, Šerman D (1982) Isoperoxidases-early indicators of somatic embryoid differentiation in pumpkin tissue. *Acta Bot Croat* 41:33–39
- Kurtar ES, Balkaya A, Kandemir D (2016) Screening for salinity tolerance in developed winter squash (*Cucurbita maxima*) and pumpkin (*Cucurbita moschata*) lines. *Int J Agric Sci* 26(2): 183–195
- Kurtar ES, Ahmet B, Dilek K (2017) Determination of semi-lethal (ld50) doses for mutation breeding of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.). *Fres Envir Bull* 26(5):3209–3216
- Kwack NC, Fujieda K (1987) Seed abortion and techniques for obtaining hybrids in interspecific crosses of *Cucurbita*. *J Jpn Soc Hortic Sci* 55(4):455–460. <https://doi.org/10.2503/jjshs.55.455>
- Lebeda A, Widrlechner MP, Staub J et al (2007) *Cucurbits* (Cucurbitaceae: *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.). In: Singh RJ (ed) Genetic resources, chromosome engineering and crop improvement, Vegetable Crops, vol 3. CRC Press, Boca Raton, FL, pp 271–376. <https://doi.org/10.1201/9781420009569.ch8>
- Lee YH, Jeon HJ, Hong KH, Kim BD (1995) Use of random amplified polymorphic DNA for linkage group analysis in an interspecific cross hybrid F₂ generation of *Cucurbita*. *J Kor Soc Hortic Sci* 36:323–330

- Lee JM, Kubota C, Tsao SJ et al (2010) Current status of vegetable grafting: diffusion grafting techniques, automation. *Sci Hortic* 127:93–105. <https://doi.org/10.1016/j.scienta.2010.08.003>
- Leelaprakash G, Rose JC, Gowtham BM et al (2011) *In vitro* antimicrobial and antioxidant activity of *Momordica charantia* leaves. *Pharmacophore* 2(4):244–252
- Li XZ, Liu JP, Zhang ZL (1994) Mutation in *Cucurbita moschata* induced by gamma-ray radiation. *J Agric Sci* 10(2):55–56
- Lima MS, Cardoso AII, Verdial MF (2003) Plant spacing and pollen quantity on yield and quality of squash seed. *Hortic Bras* 21:443–447. <https://doi.org/10.1590/S0102-05362003000300005>
- Lira R, Tellez O, Davila P (2009) The effects of climate change on the geographic distribution of Mexican wild relatives of domesticated Cucurbitaceae. *Genet Resour Crop Evol* 56(5):691–703. <https://doi.org/10.1007/s10722-008-9394-y>
- Lopez-Bucio J, de la Vega OM, Guevara-Garcia A et al (2000) Enhanced Phosphorous Uptake in Transgenic Tobacco Plants that Overproduce Citrate. *Nature Biotech* 18:450–455
- Loy B (2012) Breeding squash and pumpkins. In: Wang YH, Behera TK, Kole C (eds) Genetics, genomics and breeding of cucurbits. Science Publishers, pp 107–110
- Marie AK, Moualla MY, Boras MG (2012) Heterosis study of some quantity characters of squash (*Cucurbita pepo* L.). *Damascus J Agric Sci* 28(1):339–354
- Mario H, Bill M, Jason S et al (1997) Oregon state University Western Oregon squash irrigation guide, vol. 541. Department of Bio resource Engineering, 116 Gilmore Hall, Corvallis, pp 737–6304
- Martha R, Gutierrez P (2016) Review of *Cucurbita pepo* (Pumpkin) its photochemistry and pharmacology. *Med Chem* 6(1):12–21. <https://doi.org/10.4172/2161-0444.1000316>
- Maxted N, Ford-Lloyd BV, Hawkes JG (2013) Plant genetic conservation: the in-situ approach. Springer Science and Business Media
- Melki M, Marouani A (2009) Effects of gamma rays irradiation on seed germination and growth of hard wheat. *Environ Chem Lett* 8:307–310. <https://doi.org/10.1007/s10311-009-0222-1>
- Metwally EIA (1985) Inheritance studies on squash crop. Ph. D. Thesis, Faculty of Agriculture, Tanta University, Egypt
- Metwally ER, Khalil RM, El-Sawy BL (1988) Genetic analysis of seed yield and related traits in summer squash (*Cucurbita pepo* L.). *Menoufia J Agri Res* 13(1):431–442
- Michael NV, Moon P, Fu F et al (2019) Genetic diversity among accessions of *Cucurbita pepo* resistant to *Phytophthora* crown rot. *HortScience* 54(1):17–22. <https://doi.org/10.21273/HORTSCI113506-18>
- Min ZY, Li H, Zou T et al (2016) Studies of *in vitro* culture and plant regeneration of unfertilized ovary of pumpkin. *Chin Bull Bot* 51(1):74–80
- Mohamed MF (1996) Phenotypic variability and selection for predominant pistil late flower expression in zucchini-type summer squash (*Cucurbita pepo* L.). *Eskandarani. First Egyptian-Hungarian Horticultural Conference*, Sept 1996, Vol 2, Kafr El-sheikh, Tanta Univ, Egypt, pp 154–162
- Mohamed SE, Abdein MA, Hikal DM (2018) Molecular genetic polymorphism, morphological and the effect of peels as natural antioxidants in some squash cultivars. *Researcher* 10(3):97–104. <https://doi.org/10.7537/marsrsj100318.11>
- Mohan NB, Madalageri MB, Ravindra HG et al (2012) Hybrid vigor and inbreeding depression in ash gourd (white pumpkin). *Plant Arch* 12(2):749–752
- Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Soria E (2005) Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol* 60:174–182
- Montero-Pau J, Blanca J, Esteras C et al (2017) An SNP-based saturated genetic map and QTL analysis of fruit-related traits in Zucchini using genotyping-by-sequencing. *BMC Genomics* 18(1):94
- Montes-Hernandez S, Eguiarte LE (2002) Genetic structure and indirect estimates of gene flow in three taxa of *Cucurbita* (Cucurbitaceae) in Western Mexico. *Am J Bot* 89:1156–1163. <https://doi.org/10.3732/ajb.89.7.1156>

- Moon P, Fu Y, Meru G (2019) Genetic diversity among accessions of *Cucurbita pepo* resistant to Phytophthora crown rot. *HortScience* 54(1):17–22
- Murrell PL, Buckler ES, Ross-Ibarra J (2012) Crop genomics: advances and applications. *Nat Rev Genet* 13(2):85–96
- Müller C, Bracher F (2015) Determination by GC-IT/MS of phytosterols in herbal medicinal products for the treatment of lower urinary tract symptoms and food products marketed in Europe. *Planta Med* 81:613–620
- Mulualem T, Abate M (2016) Heterotic response in major cereals and vegetable crops. *Int J Plant Breed Genet* 10:69–78. <https://doi.org/10.3923/ijpb.2016.69.78>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Phys Plant* 15(3):473–497
- Murkovic M, Müllleder U, Neunteufl H (2002) Carotenoid content in different varieties of pumpkins. *J Food Compos Anal* 15(6):633–638. <https://doi.org/10.1006/jfca.2002.1052>
- Murovec J (2015) Phenotypic and genetic diversity in pumpkin accessions with mutated seed coats. *Hort Sci* 50(2):211–217
- Nabhan GP (1985) *Gathering the desert*. University of Arizona Press, Tucson
- Napier T (2009) Pumpkin production. *Primefacts* 964:1–8
- Nascimento WM, Pinheiro F, Freitas RA (2007) Evaluation of lettuce seed physiological quality under adverse temperatures. *Rev Bras Sement* 29(3):175–179
- Ndoro OF, Madakadze RM, Kageler S, Mashingaid AB (2007) Indigenous knowledge of the traditional vegetable pumpkin (*Cucurbita maxima/moschata*) from Zimbabwe. *Afr J Agric Res* 2:649–655
- Neel R, Vandana, Najibullah M et al (2017) A review on *Cucurbita pepo*. *Int J Pharm Phytochem Res* 9(9):1190–1194. <https://doi.org/10.25258/phyto.v9i09.10305>
- Ntulia NR, Tongoanab PB, Zoboloa AM (2015) Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. *Sci Hortic* 189:192–200. <https://doi.org/10.1016/j.scienta.2015.03.020>
- Nuez F, Fernandez de Cordova P, Ferriol M et al (2000) *Cucurbita* ssp. and *Lagenaria siceraria* collection of the genebank of the center for conservation and breeding of the agricultural biodiversity (COMAV) of the Polytechnical University of Valencia. *Cucurbit Genet Coop Rep* 23:60–61
- Obi RK, Nwanebu FC, Ndubuisi UU et al (2009) Antibacterial qualities and phytochemical screening of the oils of *Cucurbita pepo* and *Brassica nigra*. *J Med Plants Res* 3(5):429–432
- Obiadalla AHA (2006) Heterosis and nature of gene action for earliness and yield components in summer squash (*Cucurbita pepo* L.). *Assiut J Agric Sci* 37:123–135
- OECD (2016) Squashes, pumpkins, zucchinis and gourds (*Cucurbita* species). In safety assessment of transgenic organisms in the environment, OECD Consensus Documents, OECD Publishing: Paris, France, Vol 5, pp 83–149
- Olson ME (2003) Stem and leaf anatomy of the arborescent Cucurbitaceae *Dendrosicyos socotrana* with comments on the evolution of pachycauls from lianas. *Plant Syst Evol* 239:199–214. <https://doi.org/10.1007/s00606-003-0006-1>
- Pal SP, Alam I, Anisuzzaman M et al (2007) Indirect organogenesis in summer squash (*Cucurbita pepo* L.). *Turk J Agric For* 31(1):63–70
- Paris HS (1996) A proposed sub specific classification for *Cucurbita pepo*. *Phytologia* 61:133–138
- Paris HS, Padley LD (2014) Gene list for *Cucurbita* species. *Cucurbit Genet Coop Rep* 37:1–14
- Paris HS, Yonah N, Portnoy V et al (2003) Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theor Appl Genet* 106:971–978. <https://doi.org/10.1007/s00122-002-1157-0>
- Patel SP, Bharodia PS, Kakade DK (2010) Concept of general and specific combining ability in relation to diallel crossing system. *Int J Agric Sci* 6(1):135–137
- Perez Gutierrez RM (2016) Review of *Cucurbita pepo* (Pumpkin) its Phytochemistry and Pharmacology. *Med Chem* 6:12–21. <https://doi.org/10.4172/2161-0444.1000316>

- Phillips KM, Ruggio DM, Ashraf-Khorassani M (2005) Phytosterol composition of nuts and seeds commonly consumed in the United States. *J Agric Food Chem* 53(24):9436–9445. <https://doi.org/10.1021/jf051505h>
- Pourcel C, Salvignol G, Vergnaud G (2005) CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA and provide additional tools for evolutionary studies. *Microbiology* 151:653–663
- Puri M, Kaur I, Kanwar RK et al (2009) Ribosome inactivating proteins (RIPs) from *Momordica charantia* for anti-viral therapy. *Curr Mol Med* 9(9):1080–1094. <https://doi.org/10.2174/156652409789839071>
- Ramon A, Torres-Ruiz RA, Hemleben V (1991) Use of ribosomal DNA spacer probes to distinguish cultivars of *Cucurbita pepo* L. and other Cucurbitaceae. *Euphytica* 53:11–17
- Rathore NS, Rai MK, Phulwaria et al (2014) Genetic stability in micropropagated *Cleome gynandra* revealed by SCoT analysis. *Acta Physiol Plant* 36:555–559 doi:<https://doi.org/10.1007/s11738-013-1429-0>
- Ratnam N (2017) A review on *Cucurbita pepo*. *Int J Pharm Phytochem Res* 9:1190–1194. <https://doi.org/10.4172/2161-0444.1000316>
- Reitsma KR, Block CC, Clark LD (2014) Cucurbit germplasm collections at the North Central Regional Plant Introduction Station Conference: Proceedings Cucurbitaceae 2014, Bay Harbor, Michigan, USA, Cucurbitaceae, pp 125–128
- Robinson RW (2008) Rationale and methods for producing hybrid cucurbit seed. *J New Seeds* 1:1–47. https://doi.org/10.1300/J153v01n03_01
- Rouphael Y, Colla G (2009) The Influence of drip Irrigation or subirrigation on zucchini squash grown in closed-loop substrate culture with high and low nutrient solution concentrations. *HortScience* 44(2):306–311
- Ruiz JM, Belakbir A, Lopez Cantarero I et al (1997) Leaf macronutrient content and yield in grafted melon plants. A model to evaluate the influence of rootstock genotype. *Sci Hortic* 71:227–234. [https://doi.org/10.1016/S0304-4238\(97\)00106-4](https://doi.org/10.1016/S0304-4238(97)00106-4)
- Saboo S, Thorat P, Tapadiya G et al (2013) Ancient and recent medicinal uses of Cucurbitaceae family. *Int J Ther Appl* 9:11–19
- Sabudak T (2007) Fatty acid composition of seed and leaf oils of pumpkin, walnut, almond, maize, sunflower and melon. *Chem Nat Compd* 43(4):383–384. <https://doi.org/10.1007/s10600-007-0163-5>
- Sáez C, Martínez C, Montero-Pau J et al (2020) A Major QTL Located in chromosome 8 of *Cucurbita moschata* is responsible for resistance to tomato leaf curl New Delhi virus. *Front Plant Sci* 11:207. <https://doi.org/10.3389/fpls.2020.00207>
- Salehi B, Sharifi-Rad J, Capanoglu E et al (2019) *Cucurbita* plants: from farm to industry. *Appl Sci* 9(3387):1–21. <https://doi.org/10.3390/app9163387>
- Sanin OG, Burbano LVB, Narvaez GAO et al (2014) Inbreeding and gene action in butternut squash (*Cucurbita moschata*) seed starch content. *Rev Fac Nal Agr Medellin* 67(1):7169–7175. <https://doi.org/10.15446/rfnam.v67n1.42634>
- Sanín OG, Restrepo MPV, Alirio F et al (2015) Genetic correlations and path analysis in butternut squash *Cucurbita moschata* Duch. *Rev Fac Nal Agr Medellín* 68(1):7399–7409. <https://doi.org/10.15446/rfnam.v68n1.47827>
- Santos MH, Rodrigues R, Gonçalves LS et al (2012) Agrobiodiversity in *Cucurbita* spp. landraces collected in Rio de Janeiro assessed by molecular markers. *Crop Breed Appl Biotechnol* 12:96–103. <https://doi.org/10.1590/S1984-70332012000200001>
- Schuster W, Haghdadi MR, Michael J (1974) Inbreeding and heterosis in oil pumpkin, *Cucurbita pepo* L. Effect of inbreeding *Zeitschrift für pflanzen zuchtung* 73(1/4):112–124 Institut Für pflanzenbau Und Pflanze Zuchtung, Justus Liebig Univeristat, Giessen, German Fedral Republic
- Sevengor S (2010) Investigations on antioxidant enzyme activities under *in vitro* and *in vivo* conditions to obtain salt tolerance in squash (*Cucurbita pepo* L.). Doctoral Thesis (Unpublished). Graduate School of Natural and Applied Sciences, Ankara University, Ankara, Turkey, pp 179

- Sevengor S, Yasar F, Kusvuran S et al (2011) The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling. *Afr J Agric Res* 6(21):4920–4924
- Shalaby TA (2007) Factors affecting haploid induction through in vitro gynogenesis in summer squash (*Cucurbita pepo* L.). *Sci Hort* 115(1):1–6
- Singh DK, Ram HH (2001) Characterization of indigenous germplasm lines of cucumber (*Cucumis sativus* L.) through SDS-PAGE. *Veg Sci* 28(1):22–23
- Sinnott EW, Durham GB (1922) Inheritance in the summer squash. *J Hered* 13:177–186
- Sisko M, Ivancic A, Bohanec B (2003) Genome size analysis in the genus *Cucurbita* and its use for determination of interspecific hybrids obtained using the embryo rescue technique. *Plant Sci* 165:663–669. [https://doi.org/10.1016/S0168-9452\(03\)00256-5](https://doi.org/10.1016/S0168-9452(03)00256-5)
- Soltysiak U, Kubicki B (1988) Induced mutations in the cucumber (*Cucumis sativus* L.). VII short hypocotyl mutant. *Genetica-Polonica* 29(34):315–321
- Spataro G, Negri V (2013) The European seed legislation on conservation varieties: focus, implementation, present and future impact on landrace on farm conservation. *Genet Resour Crop Evol* 60:2421–2430. <https://doi.org/10.1007/s10722-013-0009-x>
- Sprague GF, Tatum LA (1942) General vs specific combining ability in single crosses of corn. *J Am Soc Agron* 34:923–952
- Stevenson DG, Eller FJ, Wang L et al (2007) Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J Agric Food Chem* 55(10):4005–4013. <https://doi.org/10.1021/jf0706979>
- Subrahmanyam NS (2004) Modern plant taxonomy. Vikas Publishing House Pvt Ltd, New Delhi, India, pp 316–321
- Tamilselvi NA, Jansirani P, Pugalendhi L (2015) Estimation of heterosis and combining ability for earliness and yield characters in pumpkin (*Cucurbita moschata* Duch. Ex. Poir). *Afr J Agric Res* 10(16):1904–1912. <https://doi.org/10.5897/AJAR2014.9099>
- Thompson HC, Kelly WC (1957) Vegetable crops. McGraw Hill Book Company, New York
- Tricoli DM, Carney KJ, Russell PF et al (2002) Transgenic plants expressing DNA constructs containing a plurality of genes to impart virus resistance. United States Patent 6(337):431
- Tsivelikas AL, Koutita O, Anastasiadou A et al (2009) Description and analysis of genetic diversity among squash accessions. *Braz Arch Biol Technol* 52(2):271–283. <https://doi.org/10.1590/S1516-89132009000200003>
- Ukiya M, Akihisa T, Yasukawa K et al (2002) Anti-Inflammatory and Anti-Tumor-Promoting Effects of Cucurbitane Glycosides from the Roots of *Bryonia dioica*. *J Nat Prod* 65(2):179–183. <https://doi.org/10.1021/np010423u>
- Wang Y, Wang C, Han H et al (2020) Construction of a high-density genetic map and analysis of seed related traits using specific length amplified fragment sequencing for *Cucurbita maxima*. *Front Plant Sci* 10:1782. <https://doi.org/10.3389/fpls.2019.01782>
- Weeden NF (1984) Isozyme studies indicate that the genus *Cucurbita* is an ancient tetraploid. *Rep Cucurbit Genet Coop* 7(37):84–85
- Weeden NF, Robinson RW (1990) Isozyme studies in *Cucurbita*. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of the Cucurbitaceae. Cornell University Press, Ithaca, New York, pp 51–59
- Wehner TC, Robinson RW (1991) A brief history of the development of cultivars in the U.-S. Cucurbit Genetics Cooperative Report 14:1–4
- Whitaker TW, Davis GN (1962) Cucurbits-botany, cultivation and utilization. Leonard Hill Ltd, London, UK, p 249
- Whitaker TW, Flory WS (1955) *Tulbagnia violacea*. Description, cultural and cytological observation. *Plant Life (Herberia)* 11:65–67
- Whitaker TW, Robinson RW (1986) Squash breeding. In: Basset MJ (ed) Breeding vegetable crops. Avi Publishing Company, Westport, pp 209–242
- Whitwood WN, Weigle JL (1978) Compact mutations in *Cucurbita pepo* L. induced by ethyl methane sulfonate. *Cucurbit Genet Coop* 1:34

- Wiedenheft B, Sternberg SH, Doudna JA (2012) RNA-guided genetic silencing systems in bacteria and archaea. *Nature* 482:331–338
- Williams MG, Davis A, Connor NO (2006) Inhibition of testosterone-induced hyperplasia of the prostate of sprague-dawley rats by pumpkin seed oil. *J Med Food* 9(2):284–286. <https://doi.org/10.1089/jmf.2006.9.284>
- Winkler C, Wirleitner B, Schennach H et al (2005) Extracts of pumpkin seeds suppress stimulated peripheral blood mononuclear cells in vitro. *Am J Immunol* 1(1):6–11. <https://doi.org/10.3844/ajisp.2005.6.11>
- Xanthopoulou A, Ganopoulos I, Kalivas A et al (2015) Comparative analysis of genetic diversity in Greek Genebank collection of summer squash (*Cucurbita pepo*) land-races using start codon targeted (SCoT) polymorphism and ISSR markers. *Aust J Crop Sci* 9(1):14–21
- Xanthopoulou A, Montero-Pau J, Mellidou I et al (2019) Whole-genome resequencing of *Cucurbita pepo* morphotypes to discover genomic variants associated with morphology and horticulturally valuable traits. *Horti Res* 6:94. <https://doi.org/10.1038/s41438-019-0176-9>
- Xiang C, Duan Y, Li Het al (2018) A high-density EST-SSR-based genetic map and QTL analysis of dwarf trait in *Cucurbita pepo* L. *Int J Mol Sci* 19(10):31–40
- Yadav M, Jain S, Tomar R et al (2017) Medicinal and biological potential of pumpkin: an updated review. *Nutr Res Rev* 23(2):184–190. <https://doi.org/10.1017/S0954422410000107>
- Yongan C, Bingkui Z, Enhui Z et al (2002) Study on affinity of sexual hybridization between *Cucurbita maxima* D. and *Cucurbita moschata* D. *Cucurbit Genet Cooperate Rep* 25:54–55
- Zagorcheva L, Alexandrova M, Aleksandrova M (1985) Genetically conditioned differences in radiation sensitivity in *Cucumis sativus* L. proceedings of the IIIrd Eucarpia meeting on breeding of cucumbers and melons proceedings of the IIIrd Eucarpia meeting on breeding of cucumbers and melons, 2–5 July, 1984, Plovdiv, 1985, pp 34–39
- Zhang Q, Yu E, Medina A (2012) Development of advanced interspecific-bridge lines among *Cucurbita pepo*, *C. maxima*, and *C. moschata*. *Hortic Sci* 47(4):452–458. <https://doi.org/10.21273/HORTSCI.47.4.452>
- Zhong YJ, Zhou YY, Li JX et al (2017) A high-density linkage map and QTL mapping of fruit-related traits in pumpkin (*Cucurbita moschata* Duch.). *Sci Rep* 7(1):1–12
- Zou T, Song H, Chu X et al (2020) Efficient induction of gynogenesis through unfertilized ovary culture with winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.). *Sci Hortic* 264:109–152
- Zraidi A, Stift G, Pachner M et al (2007) A consensus map for *Cucurbita pepo*. *Mol Breed* 20:375–388