International Journal of Plant Breeding ***©2007 Global Science Books***

Breeding Pumpkin (*Cucurbita moschata* Duch. ex Poir.) for Fruit Yield and Other Characters

Pranab Hazra[1*] • Alok Kumar Mandal[2] • Avijit Kumar Dutta[1] • Hari Har Ram[3]

[1] Department of Vegetable crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252, West Bengal, India
[2] Department of Horticulture, Govt. of West Bengal, India
[3] Krishidhan Seeds Pvt. Ltd., Pune, India
Corresponding author**: * hazrap@rediffmail.com**

ABSTRACT

Pumpkin (*Cucurbita moschata* Duch. ex Poir.) with its origin in the north western part of South America is the most important and extensively cultivated cucurbit in India, Africa, Latin America, Southern Asia and the United States and occupies a prominent place among the vegetables owing to its high productivity, nutritive value, good storability, long period of availability and better transport potentialities. This review article orients on origin and taxonomy, breeding behaviour, inheritance of important characters, breeding methods and biotechnological applications in breeding pumpkin for fruit yield and quality.

Keywords: *Cucurbita* species, genetic diversity, hybrid, origin, resistance, tissue culture

CONTENTS

INTRODUCTION

The genus *Cucurbita* incorporating pumpkin, squashes or vegetable marrows is the most economically valuable genus of this family with 27 species, five of which (*C. moschata*, *C. pepo*, *C. maxima*, *C. mixta*, *C. ficifolia*) are cultivated all

Received: 24 December, 2006. Accepted: 10 February, 2007.

Invited Review

over the tropical and sub-tropical world. The term "squash" is generally employed to designate the forms of *C. pepo*, that are used immature, all baking cultivars of *C. maxima* and the cushaw-type cultivars of *C. mixta*, used mature and the term "pumpkin" is normally applied to the edible fruit of any species of *Cucurbita* utilized when ripe as a table vegetable or in pie (Whitaker and Robinson 1986). However, pumpkin is mostly associated with *Cucurbita moschata* Duch. ex Poir. It is extensively cultivated in India, Africa, Latin America, Southern Asia and United States. However, the English name for the species, "tropical pumpkin" is an appropriate one because the greatest diversity lies in the neotropics where the vines are grown under a wide range of ecological conditions, including under hotter conditions than are tolerated by the other cultivated *Cucurbita* species (Andres 2004). "Butternut squash" is also popularly called pumpkin in western countries. Pumpkin now occupies a prominent place among the vegetables owing to its high productivity, nutritive value, good storability, long period of availability and better transport potentialities. Pumpkin, like the majority of the domesticated *Cucurbita* species is monoecious and exhibits a succession of flower development, with a decreasing ratio of staminate to pistillate flowers as the plants mature. Flowers are axillary, solitary, unisexual, large and showy. The edible portion of the fruit, botanically called 'pepo', is a pericarp with a very little portion of the mesocarp. Both mature and immature stages of the fruits are used as vegetable. The flesh is delicious when stewed, boiled or baked. The average nutritive value of pumpkin (2.68) is higher than brinjal (2.41), tomato (2.09) and cucumber (1.69) mainly because of high stored carbohydrate (mainly glucose) and carotenoids (74% β-carotene) along with a moderate quantity of ascorbic, nicotinic, pantothenic and folic acids and different minerals (Gupta and Rai 1990). It is particularly important for the supply of antioxidants and especially carotenoids in foods. Matured fruits are also used as industrial raw material for carotene production (Vucetic *et al.* 1989). Fully matured fruits, apart from utilization as a cooked vegetable can be used in preparing sweets, candy or fermented into beverages. Delicate sweet items like "halwa", other sweets and jams are prepared from the meshed flesh of fully matured fruit. Pulp is also mixed with tomato in the preparation of sauce and ketchup (Sharma and Kumar 1995). It is also used for the preparation of GTF (glucose tolerance factor) pumpkin milk powder which can be used as a diabetic food and pumpkin ice cream. The fermented product "Yerusseri" prepared from immature fruit is very popular in Kerala, India (Rajan and Markose 2001). Its young leaves, tender twigs and flowers are also used as a cooked vegetable in different forms. This review serves to introduce to the readers different aspects related to breeding pumpkin.

ORIGIN AND TAXONOMY

The Cucurbitaceae is a tropical or semitropical family to which the genus *Cucurbita* belong. The *Cucurbita* genus, indigenous to the new world is a comparatively closed group or system of about 27 species. Cytogenetically, the species of *Cucurbita* show amazing uniformity in chromosome number and all the species have 20 pairs (2n = 40) of small, dot like chromosomes (Whitaker and Robinson 1986) and isozyme studies indicate the allotetraploid origin of the genus (Weeden 1984; Kirkpatrick *et al.* 1985). Sisko *et al.* (2003) analyzed the genome size of 11 *Cucurbita* species and found that in contrast to the uniform chromosome number, the genome size differences within *Cucurbita* genus as measured by flow cytometry based on propidium iodide appeared to be large, 2C values ranging from 0.686 pg in *C. foetidissima* to 0.933 in *C. ficifolia* and the genome sizes of the five cultivated species were 0.864 pg (*C. pepo*), 0.887 pg (*C. maxima*), 0.933 pg (*C. ficifolia*), 0.708 pg (*C. moschata*) and 0.748 pg (*C. mixta*). Morphological differentiation of different species appears to be based upon gene mutation, rather than differences in chromosome numbers and polyploidy (Whitaker and Robinson 1986). The five cultivated species *C. pepo*, *C. moschata*, *C. mixta, C. maxima* and *C. ficifolia* were selected by American Indians long before the discovery of America (Whitaker and Bemis 1976). Archaeological remains trace the association of pumpkins and squashes with man in America for at least 10,000 years (Whitaker and Cutler 1971). It was presumed that fruits of most wild species of *Cucurbita* were of a size and colour to attract the attention of primitive men but they had hard, tough rinds and excessively bitter flesh but the seeds were non-bitter, tasty and nutritious and the early men, in sampling the fruits of wild *Cucurbita* species for seeds, probably found a lack of the bitter principle which resulted in the modern domesticated species (Whitaker and Bemis 1976). Temporal and geographical relationships among bean, squash, gourds and humans in eastern North America suggested the possibility of a long-term genetic interaction between wild and domesticated species (Kirkpatrick and Wilson 1988). The centre of diversity of *Cucurbita* is in the area south of Mexico City extending to the Mexico-Guatemala border (Whitaker and Bemis 1976), and South Central Mexico is believed to be the centre of distribution (Whitaker and Knight (Jr.) 1980). Not surprisingly, the wild species, *C. lundelliana* and *C. martinezii*, which are evidently closely related to the cultivated species, occur in this area (Whitaker and Robinson 1986). Native areas of the cultivated species of *Cucurbita* appear to be North Mexico of Mexico City for *C. pepo*, South America for *C. maxima*, South America, Central America and Mexico for *C. moschata*, Mexico and South Mexico City for *C. mixata* and Central America and Mexico for *C. ficifolia* (Hurd *et al.* 1971).

Wild forms of *C. moschata* have not been described, but primitive appearing landraces are known from Central America to northern Peru which, coupled with archaeological evidence, suggests that the center of origin of pumpkin is in northwestern South America (Andres 2004). Molecular analysis using AFLP and SRAP markers showed a genetic diversity concordant with the morphological variability and with both the markers, the accessions clustered according to geographical origin: Central America, South America and Spain suggesting the existence of two independent domestications in both American areas and/or introgressions from related species (Ferriol *et al.* 2004).

C. moschata appears to be the possible common ancestor of the genus *Cucurbita* and the five cultivated species namely *C. pepo*, *C. moschata*, *C. maxima*, *C. mixata* and *C. ficifolia* came out independently as separate entities (Hurd *et al.* 1971; Nee 1990). Comparison of DNAs of 20 *Cucurbita* species showed no difference in their banding following CsCl configuration, however, DNA hybridisation experiments among nine species indicated that *C. palmate* is distant to other species, while *C. lundelliana*, *C. maxima* and *C. pepo* are closely related (Goldberg *et al.* 1972). Phylogenetic analysis indicated that *C. moschata* and *C. mixata* are probably sister species and that *C. pepo* shares a common ancestor with these species that is not shared with *C. maxima* (Decker-Walters *et al.* 1990). Cladistic analysis of 86 chloroplast DNA restriction-site mutations among 30 samples representing 15 species of *Cucurbita* indicated that annual species of the genus are derived from perennials. The Malabar Gourd, *C. ficifolia* is placed as a basal, sister taxon relative to other domesticated species and allied wild types. The pattern of variation supports three species groups as monophyletic: (1) *C. fraterna*, *C. pepo* and *C. texana*, (2) *C. lundelliana*, *C. martinezii*, *C. mixta*, *C. moschata* and *C. sororia*, and (3) *C. foetidissima* and *C. pedatifolia*. Domesticated samples representing subspecies of *C. pepo* are divided into two concordant groups, one of which is allied to wild types referable to *C. texana* and *C. fraterna*. The data failed to resolve relationships among cultivars of *C. moschata* and *C. mixta* and their association to the wild C. *sororia*. The South American domesticate *C. maxima*, and its companion weed, *C. andreana* show close affinity and alliance to *C. equadorensis* (Wilson *et al.* 1992). Most of the domesticated *Cucurbita* species are associated with the closely related wild or weedy species revealing gene flow between domesticated and wild, weedy forms of *Cucurbita* species and indicating the possibility of introgressive hybridization (Wilson 1990).

CHARACTERIZATION OF CULTIVATED *CUCURBITA* SPECIES

The chief species identifying characters of *C. moschata*, *C. pepo*, *C. maxima*, *C. ficifolia* and *C. mixata* according to Whitaker and Davis (1962) are:

- Plant perennial, seeds black or tan: *C. ficifolia*;
- Plants annual; seeds white, buff or tawey: four species, *C. moschata*, *C. pepo*, *C. maxima and C. mixata*;
- Peduncle hard, smoothly grooved, flared at fruit attachment; foliage non-stipulate: *C. moschata*;
- Stems hard, angular; peduncle angular, grooved; peduncle hard, sharply angular, grooved, foliage stipulate: *C. pepo*;
- Plants annual; seed white, buff or towey; stem soft, round; peduncles soft, terete, enlarged by soft cork: *C. maxima*;
- Peduncle hard, greatly enlarged in diameter by hard cork, not flared at fruit attachment; foliage non-stipulate: *C. mixta*.

Botanical descriptions of pumpkin (*C. moschata*), summer squash (*C. pepo*) and winter squash (*C. maxima*) after Whitaker and Davis (1962) and Chakravarty (1982) are presented in **Table 1**.

The globular, elongated and acorn-shaped ovary always give rise the respective fruit shape but an oval-shaped ovary may result in either an oval or flattened fruit shape (Mandal 2006).

FLOWERING AND INTERSPECIFIC CROSSABILITY

Sex expression in *Cucurbita* is relatively stable and all species including *C. moschata* are monoecious. Flowers are unisexual, solitary in the leaf axils. Generally stalks of the staminate flowers are longer (3-10 cm) and thinner (0.50-0.75 cm) than that of the pistillate flowers. The stalk of the pistillate flowers is distinctly angular and longitudinally furrowed, 2-6 cm long and 0.75-1.0 cm thick. Like that of other monoecious cucurbits, generally staminate flowers appear before the pistillate flowers in pumpkin. However, few genotypes show a reverse trend by producing the pistillate flowers first, particularly under short day and cool temperature of 30.1°/17.4°C day/night (Mandal 2006). There was much variation in the ratio of staminate: pistillate flowers (4:1 to 20:1) in the vine and staminate flowers opened after 55 to 70 days, pistillate flowers after 60-75 days of seed sowing (Mandal 2006). The staminate and pistillate flowers are of about equal size. In the staminate flowers the filaments are free, but the anthers are united into a more or less column like mass. Nectar is produced by both staminate and pistillate flowers. The heavy and sticky pollen grains from the staminate flowers are generally transported by domestic honey bees to the receptive stigma of the pistillate flower to accomplish fertilization. Record of differential crossing success of pumpkin with other *Cucurbita* species as per Rai *et al.* (2006) is presented in **Table 2**.

DIVERSITY IN DIFFERENT CHARACTERS OF PUMPKIN

General character diversity recorded from the assemblage of different Indian materials (Sirohi 1993; Mandal 2006) is presented in **Table 3**.

Table 1 Botanical characters of pumpkin, summer squash and winter squash.

Characters	***C. moschata***	***C. maxima***	***C. pepo***
Growth habit	Annual vine	Annual vine	Annual vine or bush
Sex expression	Monoecious	Monoecious	Monoecious
Foliage texture	Soft, hairy	Foliage not harsh or prickly, bearing small setate interspersed with soft hairs	Stiff, upright, harsh and prickly to touch
Foliage shape	5-6 shallowly lobed, lobes acute or rarely obtuse, often with whitish blotches on the upper surface.	Leaves more or less reniform with 5 rounded, shallow lobes, margin minutely dented	Leaves broad, obtuse or acute, usually with 5 deep lobes, margin dentate, upper surface glabrous and lower with glandular club shaped hairs
Tendril	Tendril many fid	Tendril 2-6 fid	Tendril branched
Flowers	Solitary	Solitary	Solitary
Calyx	Calyx tube of staminate flower short or lacking, lobes 3, linear, very often leafy	Calyx tube club shaped, lobes 5, short, linear and hairy	Calyx tube obscurely 5 angled
Flower	Corolla campanulate, pale yellow, mostly reflexed, lobes 5, stamens 3, filaments swollen at the base, glanduliform	Corolla campanulate, light to deep yellow, lobes 5, reflexed, stamens 3, filaments thick, glanduliform	Corolla campanulate with erect or spreading lobes, stamens 3, filaments swollen below
Peduncle	Smooth, 5 angled expanded or flared at fruit attachment	Spongy, cylindrical, soft, corky	5 angled with little or no expansion at fruit attachment
Fruits	Usually large of variable shape and size, globular, cylindrical, flattened	Usually large, oblong- cylindrical or flattened-cylindrical	Usually of variable size, shape and colour
Seeds	Yellow with a thin or ragged margin, scalloped or shredded in appearance, the margin more deeply coloured than the body of the seed	Seed white or yellowish with margins of different colour	Seeds whitish yellow broadly or narrowing ovate usually with raised, smooth, well-differentiated margin

Table 2 Interspecific crossability among different *Cucurbita* species.

Species	(1)	(2)	(3)	(4)	(5)	(6)
C. moschata (1)	SC	***	*	*	***	CC(*)
C. pepo (2)	***	SC	***	*	*	CC +
C. mixta (3)	*	***	SC	*	***	CC +
C. maxima (4)	*	*	+	SC	**	CC (x)
C. ficifolia (5)	*	**	***	**	SC	CC (x)
C. lundelliana (6)	CC (x)	CC +	CC +	CC (x)	CC (x)	SC

SC: self compatible; CC: cross compatible; *: F_1 viable but self sterile; **: Few viable F_1 seeds and weak F_1 plants; ***: F_1 sparingly viable and self sterile; +: small F_1 seeds and very weak seedlings; (x): self fertile F1 plant

Table 3 Diversity in different characters of pumpkin.

Stem characters	Soft hairy, harsh to some what prickly, polyangled or cylindrical
Leaf shape	Ovate, orbicular, reniform, retuse
Leaf size	Small, intermediate, large
Leaf margin	Smooth, dented
Leaf lobes	Absent, shallow, intermediate, deep
Leaf spots	White or silver, absent
Stalk length of staminate flower	(3-10 cm)
Sex ratio	5:1 to 20:1
Calyx of pistillate flower	Leaf shaped, pointed end, linear
Ovary shape	Globular, flattened, acorn, elongated, oval
Fruit shape	Globular, round, flattened, dumbell, disc, oblong, cylindrical, oval, acorn or heart shaped, pyriform, crooked neck, elongate
Fruit size index	Small, medium, large and very large
Flesh thickness	Thin, medium, thick, very thick
Flesh colour	Cream, yellowish green, yellow, orange, salmon
Fruit skin colour at immature stage	Dark green, green, dark green with whitish patches
Fruit skin colour at maturity	Green, blackish-green, cream, yellow, orange, orange-yellow, brown, mottled (dark green with orange spots, orange with dark green patches, light orange with dark green spots and patches, blackish with orange patches, dark green with creamy spots, dark green with irregular white stripe, dark green with yellow patches, blackish with irregular yellowish stripes, orange with blackish patches, orange with dark green spots
Fruit shape at stem end	Flattened, depressed, pointed
Fruit shape at blossom end	Flattened, depressed
Peduncle surface	Hard corky, soft corky
Peduncle shape	Deep-grooved, light-grooved, cylindrical
Peduncle base	Large flared, medium flared, low flared
Fruit ribs	Smooth or no ribs, superficial, intermediate deep
Flesh texture	Smooth, firm, soft spongy, fibrous, grainy
Peduncle separation from fruit	Easy, intermediate, difficult
Average fruit weight	1.5 to 40.0 kg

GENETIC DIVERGENCE OF PUMPKIN

Of the three main cultivated *Cucurbita* species, *C. moschata* is the least studied although it is the most widely cultivated *Cucurbita* in the tropics and a highly polymorphic domesticate for which different intraspecific classifications based on fruit shape, geographic origin and other characterristics have been made and whose greatest diversity lies in the neotropics (American tropics) where the vines are grown under a wide range of ecological conditions (Andres 2004). The southern region of Brazil has a great range of *Cucurbita* species especially in shape, weight, average length, skin firmness, presence of bulges and external colour of fruits and based on leaf and pedicel characteristics, it was possible to classify the accessions into three species: *C. pepo*, *C. maxima* and *C. moschata* (Choer 1999). However, peduncle characters which are important to classify accessions into these three species, did not conform to the characters of *C. moschata* strictly in some inbreds developed from indigenous open pollinated materials of India but rather to the peduncle character of *C. moschata*, *C. maxima* and *C. mixta* which was expressed conjointly in them (Mandal 2006). Most of the peduncle surface was hard corky even though soft corky, which is regarded as a character of *C. maxima* (Whitaker and Davis 1962) was also found in three inbreds. The peduncle was mostly grooved but the intensity of grooving varied (angular grooved, smoothly grooved) and even a somewhat cylindrical peduncle was recorded in five inbreds (Hazra 2006; Mandal 2006). In all the inbreds peduncles flared at fruit attachment however, flaring was very low in eleven inbreds which some what resembles *C. mixta* (Mandal 2006). These findings amply support the proposal of probable gene exchange among these three monophylatic species of *cucurbita* (Wilson *et al.* 1992) during the process of domestication and cultivar development, which is usually expected because *C. moschata* is a highly polymorphic domesticate for which different intra-specific classification based on fruit shape, geographic region and other characteristics have been made (Andres 2004). Pumpkin, after its introduction from South America by foreign navigators and emissaries has well been accepted in India and its wide-spread cultivation has caused the development of huge genetic diversity in different areas. Diversity in the Indian gene centre is concentrated in Indo-Gangetic plains, the north-eastern region, north-western Himalayas, the Western and Eastern Ghats and sporadically in the tribal dominant belt of central India (Srivastava 2006).

Determination of genetic diversity by biochemical and molecular markers

Biochemical and molecular markers are generally used to a) discriminate different *Cucurbita* species, b) analyze the amount of genetic diversity in *C. moschata* land races and c) classify the land races to assist in selection of parental genotypes for improvement of different horticultural characters. Allozyme variation could be used to characterize *Cucurbita* accessions because the pattern of allozyme variation were consistent with the variation in fruit, seed and peduncle characters that define the domesticated taxa, *C. moschata*, *C. maxima* and *C. pepo* (Wilson 1989). However, Amaral Jr. *et al.* (1999) suggested that the use of genetic markers might be more beneficial for genotype discrimination as isoenzyme polymorphisms did not correlate with morphological polymorphisms of pumpkin accessions and increasing knowledge of the molecular diversity of a crop is essential for extending its genetic base, identifying cultivars and selecting parental varieties for breeding programmes.

Random amplified polymorphic DNA (RAPD) markers have been used by several workers. Jeon *et al.* (1994) generated RAPD markers by 6 out of 50 arbitrary 10-mer primers which were effective in discriminating 9 *C. moschata* and 6 *C. pepo* cultivars. The primers produced 64 useful RAPD markers of 350 to 6000bp size. Some 11% markers were common to both *C. moschata* and *C. pepo*, whereas 62.5% were specific to one or other species and the rest were polymorphic in either or both the species. The average dissimilarity coefficient matrix of markers was 5.84 between *C. moschata* and *C. pepo*, 3.41 between *C. moschata* cultivars and 2.90 between *C. pepo* cultivars. Fifteen cultivars of *C. moschata* and *C. pepo* could be distinguished from each other by single primer or by the combination of 2 to 3 primers. In other similar study with the Chinese cultivated pumpkin and squash resources RAPD analysis clearly discriminated *C. moschata*, *C. maxima* and *C. pepo* (Liu *et al*. 2004). In this study, 9 primers were screened from 120 arbitrary 10-mer primers, and a total of 60 DNA bands were amplified, 59 of which were polymorphic. Lee *et al.* (1995) screened 70 arbitrary 10-mer primars in an F_2 population of *C. pepo* × *C. moschata* of which 15 showed polymorphism in parental DNAs and 5 linkage groups containing 28 RAPD marker loci could be constructed analyzing 47 segregating RAPD markers. Young and Chung (1998) used RAPD markers to investigate genetic relationships among 22 indigenous varieties of *C. moschata* from all regions of South Korea. Of the 35 primers used, only 11 revealed clear and reproducible banding pattern and only 16 were polymorphic among the total 86 bands. Based on the presence of polymorphic bands, the 22 varieties were clustered in three groups and close genetic relatedness to geographical distribution of the varieties was concluded from this study. Gwanama *et al*. (2000) employed RAPD markers to analyze the amount of genetic diversity in *C. moschata* land races grown in south-central Africa. Cluster analysis based on 39 polymorphic and 105 monomorphic DNA fragments amplified by 16 primers revealed 4 clusters into which all the 31 genotypes from Zambia and Malawi were grouped. The genotypes from Malawi fell in 3 clusters while all the genotypes from Zambia and three from Malawi came together in one cluster and this analysis further supported the proposition of close correspondence between genetic relatedness and geographical distribution, put forward earlier by Young and Chung (1998). RAPD markers have also been used to construct a partial map of the *Cucurbita* genome. Sequence Based Amplified Polymorphism (SBAPs), which preferentially amplify coding regions of the genome grouped the different pumpkin accessions fundamentally according to the type of use viz., human consumption, animal fodder or ornamental use, which suggested the usefulness of both types of markers for discriminating accessions of breeding interest (Ferriol *et al.* 2003). The mapping population for an experiment on RAPD marker-assisted selection consisted 162 backcross individuals from a cross between *C. pepo* × *C. moschata* cv. Nigerian Local. The BC_1 progeny segregated for bi-colour (yellow/green), peduncle colour, colour intensity, orange or white flesh, wartiness, mottled leaves, powdery mildew and zucchini yellow mosaic virus (ZYMV) susceptibility. The morphological traits associated with the RAPD markers were fruit colour (including the economically important B gene), mottled leaf and a virus resistance gene complementary to ZYMV resistance (Brown and Myers 2001).

Genetic divergence by multivariate analysis

Genetic divergence of pumpkin by different sets of multivariate analyses most of the time did not permit a correlation between genetic diversity and eco-geographic origin. The traits that contributed most to genetic dissimilarity were days to flowering, vine length, number of first female flowering node, fruit weight, number of fruits per vine and 1000-seed weight (Lakshmi *et al*. 2003); seed length, the largest fruit diameter, fruit length, node number for the first male flower, stem diameter, number of days for the first female flower, internode length, fruit weight, total soluble solids, and number of seeds per gram (Ramos *et al.* 2000); fruit weight, leaf area, seed weight per fruit, and yield per vine (Kale *et al*. 2002) and fruit weight; 100-seed weight, carotene content of the pulp, total sugar content of the pulp, peduncle length, equatorial diameter of fruit, polar diameter of fruit, fruit yield/plant, pericarp thickness and fruits per plant (Mandal 2006). Hence, selection of divergent parents based on these characters may be useful for heterosis breeding in pumpkin.

SELECTION INDICES FOR FRUIT YIELD AND QUALITY

Different studies clearly suggested fruit weight, particularly weight of the first mature fruit, along with other characters *viz*., fruits per plant, equatorial and polar diameter of fruit, pericarp thickness of fruit and days to anthesis of first pistillate flower to be the key yield components (Doijode and Sulladmath 1986; Gwanama *et al.* 1998; Kumaran *et al.* 1998; Bindu *et al.* 2000; Mohanty 2001; Mandal 2006) indicating the importance of these traits for the selection of high-yielding genotypes of pumpkin.

A wide range of total sugar content from 1.18 to 6.0% (Vucetic *et al.* 1989; Chung and Youn 1998; Chaturvedi 2001; Mandal 2006), of carotene content from 1.6 to 45.6 mg/100 g fresh pulp (Wu and Jin 1998; Danilceuko and Genyte 1999; Gwanama *et al.* 2002; Murkovic *et al.* 2002; Mandal 2006) and of ascorbic acid content from 0.83 to 5.49 mg/100 g fresh weight (Chung and Youn 1998) in different genotypes justifies the possibility of improving these quality characters of pumpkin through appropriate breeding approaches.

Some important findings like the positive correlation between β-carotene and fruit diameter as well as between vitamin C and dry matter content of fruit (Danilcenko and Genyte 1999), a positive but non-significant association between ascorbic acid and β-carotene content with fruit yield (Pandey *et al.* 2002) and no negative direct effect of both β-carotene and total sugar content of pulp on fruit yield (Mandal 2006) amply justified the possibility of simultaneous selection for sugar, ascorbic acid and carotene content of pulp without a concomitant depression in fruit yield. There were high correlations between sensory sweetness and total ethanol-soluble sugars, and between sensory texture and solids content of fruit pulp of *C. moschata* (Daniel *et al.* 1996).

INHERITANCE OF DIFFERENT CHARACTERS

The three economically important species, *C. pepo*, *C. moschata*, and *C. maxima* are highly polymorphic in fruit characteristics, inspiring much research into their inheritance although most of such studies have been done in *C. pepo* and *C. maxima*. The new gene list for pumpkin and squash includes descriptions of gene interactions and the genetic background of the parents that had been used for crossing to allow easy confirmation of previous work and provide a sound foundation for further investigation. This gene list includes 79 loci for phenotypic/morphological traits and 48 polymorphic allozyme loci along with linkage and gene mapping (Paris and Brown 2005). However, information on simply inherited characters documented in the gene list is almost lacking for *C. moschata*. and among the few that have been reported so far are Trifluralin (a herbicide) tolerance controlled by a dominant gene "*T*" (Adeoye and Coyne 1981), naked kernel (absence of tough seed coat) trait controlled by a pair of recessive genes (Zhou 1987) and mottled light and dark green fruit colour, monogenic dominant ("*Mldg*") over dark green fruit (Cardoso *et al.* 1995). A wide range of expression of the trait "silver leaf mottling" was observed in *C. moschata* although all the reports indicated that this trait showed dominance over non-mottled leaf. Ribeiro and da Costa (1989) reported that a partially dominant gene was responsible for silver mottling in leaf but modifier genes affected its expression while it was reported to be conditioned by two genes (Latha and Gopalkrishnan 1993) and a single gene "*M*" (Wessel-Beaver and Katzir 2000) in other two reports.

Information on polygenic inheritance of quantitative characters in pumpkin is also meager. Non-additive gene action for vine length, number of laterals, nodes of first staminate and pistillate flowers and days to first staminate and pistillate flowers (Doijode and Sulladmath 1988); for number of seeds/fruit, 100-seed weight and seed size index (Doijode *et al.* 1987); over-dominance (Galka 1987) and dominance (Sirohi *et al.* 1986) for fruit weight; over-dominance for vine length, fruit number/plant, flesh thickness, fruit shape index and fruit yield/plant (Sirohi *et al.* 1986) and over-dominance gene action for all the nutritional traits, namely, total soluble solids, carotenoids, ascorbic acid, calcium, and iron content (Sirohi and Yayasani 2001) clearly suggest the importance of exploiting heterosis commercially.

The sex form of pumpkin is monoecious, but ubiquitously. Identification of gynoecious sex form in *C. maxima* (Singh and Rhodes 1961) and *C. pepo* (Shifriss 1985) and a single dominant gene controlling gynoecious sex expression in *C. foetidissima* (Robinson 2000) opened a possibility of transferring this character to pumpkin, *C. moschata*.

BREEDING METHODS

The main objectives of pumpkin breeding include development of medium or short vines with high degree of female sex expression, high fruit yield, earliness as indicated by first pistillate flower at early node number, thick flesh quality of good with high β-carotene and sugar contents and resistance to important diseases and insect pests (Swarup 2006). Different breeding methods are discussed.

Individual plant selection

Pumpkin, as in other *Cucurbita* species, though cross-pollinated in nature, generally do not show significant loss in vigour due to inbreeding (Mandal 2006), and the idea that inbreeding in *Cucurbita* does not decrease vigour came from some very early studies (Haber 1928; Scott 1934).In these crops, inbreeding and individual plant selection through pedigree breeding can be practiced as effectively as in self-pollinated crops. The three important uses of inbreeding are to attain uniformity in plant characters, to improve yield by individual plant selection and to recombine suitable inbred lines. Inbred selection was utilized to develop different improved varieties of pumpkin (Pexioto *et al.* 1990; Sirohi *et al.* 1991; Hazra and Banerjee 2005). First one or two female flowers on the plant should be selfed, otherwise selfing in later stages often fail to set fruits. It was evidently clear from different studies that the heterozygous progeny of the open-pollinated cultivars segregated in successive generations produced improved genotypes for different characters. Damarany (1989) recorded considerable increase in each character, particularly fruit weight (50%) and yield (40%) and even resistance to powdery mildew after three generations of inbreeding. Koutsika-Sotiriou *et al.* (2004) also suggested three cycles of pedigree breeding and selfing to develop improved genetic stock of different *Cucurbita* species including *C. moschata*. Having acceded to this proposition, four generations of consecutive selfings and selections produced marked improvement for fruit yield/plant, marketable fruits/plant and carotene content of the pulp showing 89.4, 85.9 and 54.2% increase, respectively over the base population and moderate improvement ranging between 7.8 to 22.7% for polar and equatorial diameter of fruits, fruit weight, 100-seed weight and total sugar content of the pulp (Mandal 2006). Kubicki and Walczak (1976) much earlier could also increase the β-carotene content as much as 70% by selfing cultivars of *C. maxima* and making selections for high β-carotene content in the flesh. Early generation selections of the inbred as well as breeding lines should be made with preference given to reduced vine length, high ratio of pistillate to staminate blossoms, earliness, high fruit yield, bright-orange colour of flesh with high total solids content. However, advanced breeding lines need to be tested for total carotenoids, total sugar and ascorbic acid contents along with sensory evaluation.

A dwarf variety of pumpkin with short vines ‘Non-vine I’ has been identified which was considered to be a GA-related mutant, and the dwarf trait results from the failure of normal internode cell elongation (Cao *et al.* 2005).

Combination breeding

Controlled hybridization between selected parental lines of pumpkin followed either by selfings and pedigree breeding or open pollination and selection is also effective in developing improved varieties because as a result of gene recombination in the progeny following a cross, it is possible to possible to select desirable segregates. After crossing between the selected parents, several generations of individual plants are selected for the specific characteristics and when one of the parents is of much better horticultural type than the other, it may be desirable to backcross to the better parent for one or more generations, followed by several generations of selfing till uniformity is attained in the breeding lines and ultimately selfed or sibbed seeds from a number of selected plants are bulked (Whitaker and Robinson 1986). Huge requirement of space in the field for the evaluation of a reasonably large segregating population to identify the desirable one becomes the main practical difficulty of this breeding method.

Development of semi-bush type character

Pumpkin, *C. moschata* has good flesh quality, but its vine growth habit hinders wide cultivation especially under cover. However, Zucchini cultivars of *C. pepo* have bushy growth habit controlled by *Bu* allele (Denna and Munger 1963), but have inferior flesh quality. Bush or semi-bush pumpkin cultivars have been developed from the interspecific crosses *C. pepo* × *C. moschata* followed by backcrossings and selfings (Kwack and Fujieda 1988; Della-Vecchia 1993; Hong *et al.* 1997, 1998). F_1 hybrid from *C. pepo* cv. Zucchini × *C. moschata* cv. PM143 (vine type) had a vine type habit with many lateral branches, similar to PM143. In F_2 and backcross populations, height and lateral branch number showed continuous variation. Inviability, weakness and sterility of the interspecific hybrids made inheritance studies difficult, but it appeared that growth habit is controlled by several pairs of genes and segregation in the F_2 of short plants morphologically similar to *C. moschata* suggested that it would be possible to develop a bush-type lines in pumpkin by interspecific hybridization (Kwack and Fujieda 1988). Pumpkin, *C. moschata* lines with good field resistance to papaya ringspot potyvirus-W and resembling the preferred *C. pepo* by having a bushy growth habit, earliness and somewhat tapered cylindrical fruits were obtained by crossing the *C. pepo* F_1 hybrid Seaulmadi × *C. moschata* cv. Piramoita followed by pedigree and backcross selection (Della-Vecchia 1993). The interspecific cross *C. pepo* cv. Ford Zucchini × *C. moschata* cv. Seoulmadi was backcrossed with the *C. moschata* cultivar and the selected individuals from the BC_1F_1 populations were selfed 3 times to integrate the bushy gene and second backcrossing to the BC_1F_4 followed by 2 successive selfings of BC_2F_1 population resulted in the development of two semi-bush type pumpkin lines with good flesh quality, Wonye 401, Wonye 402 (Hong *et al.* 1997). Following the same breeding strategy two more semi-bush type pumpkin lines, Wonye 403 and Wonye 404 have been developed from the interspecific cross *C. pepo* cv. Ford Zucchini × *C. moschata* cv. Puthobag (Hong *et al.* 1998).

Transfer of precocious yellow fruit pigmentation

The ‘*B*’ gene, which conditions precocious yellow fruit pigmentation before anthesis produce deep yellow coloured fruit with high vitamin A content (Shifriss 1981). It is chlorophyll deficiency character in different plant parts and controlled by two unlinked genes, *B1* and *B2* (Shifriss 1991b). This gene from *C. pepo* was successfully transferred to *C. moschata* (Paris *et al.* 1985). Later, Shifriss *et al.* (1989) developed two different *B* lines of *C. moschata* after gene transfer from *C. pepo* and *C. maxima* viz., NJ-B (*B1* gene from *C. pepo*) and IL-B (*B2* gene from *C. maxima*). From the study of the cross NJ-B × IL-B, the two *B* gene appeared to be unlinked and a third gene to be closely linked to the *B* gene of IL-B which was thought to activate the expression of *B* gene in the stem (Shifriss *et al.* 1989). It was further recorded that expression of chlorophyll deficiency conditioned by *B* gene in all the plant parts (stem, leaf blade, petiole, midrib, pedicel and ovary) depends upon the genotype carrying this gene with the modification due to environment (Shifriss 1991a). Inheritance pattern of the *B* genes was further studied by Shifriss (1993) in the F_2 population of the *C. moschata* cross IL-B × NJ-B, where the female parent is thought to have the constitution *B1+ B1+ B2B2* and the male parent *B1B1 B2+ B2+*. The breeding programme led to the development of two phenotypically distinct lines: a) plants producing fruits in which chlorophyll deficiency affected about half their surface and b) plants producing fruits in which chlorophyll deficiency was confined to a few golden spots of relatively small area at the proximal end and three phenotypic classes were distinguished in this class: i) PDC-UO, PDC-SL, denoting precocious depletion of chlorophyll uniformly affecting ovaries before anthesis, where the subsequent expressivity level of chlorophyll deficiency becomes low and stem colour fluctuates between golden and green (phenotype is genetically *B2B2*), ii) GOT-VL, GS, denoting uniformly green ovaries at pre-anthesis, chlorophyll deficiency becomes becoming visible late in post-anthesis and being confined to a small area, often inconspicuous at the proximal end of the fruit and representing a very low expressivity of chlorophyll deficiency (phenotype is genetically *B2B2+*) and iii) GO, GS, with ovaries, fruits and stems persistently green (phenotype is genetically either *B*2+*B*2+ exclusively or a combination of *B*2+*B*2+ in various proportions).

Heterosis breeding

Absence of inbreeding depression does not signify that hybrid vigour in *Cucurbita* species is lacking. Advantages that F_1 hybrids may have over open-pollinated cultivars include increased vigour, enhanced fruit yield, earlier and more uniform maturity. A number of investigators have found significant evidence for hybrid vigour in pumpkin. Pronounced positive heterosis for earliness, fruit number, fruit weight, fruit flesh thickness and fruit yield was reported by several researchers (Lozanov 1969; Doijode *et al.* 1982; Chattopadhyay 1984; Sirohi *et al.* 1985; Mohanty and Mishra 1999; Carle *et al.* 2000; Mahajan and Sirohi 2000; Gwanama *et al.* 2001; Mohanty and Prusti 2002; Sirohi *et al.* 2002; Pandit *et al.* 2006). Variety effects and heterosis mean squares were significant for the days to anthesis of pistillate flower, the weight of the first mature fruit, the mean fruit weight and soluble solids, indicating the presence of both additive and non-additive gene actions (Gwanama *et al.* 2001). Significant positive heterosis for total soluble solids, total sugar and carotene content of the pulp in few hybrids due predominantly to additive and additive × additive epistasic component of genetic variance (Pandit *et el.* 2006) is highly encouraging, while a report of marked negative heterosis for β-carotene content (Doijode 1983) emphasized the importance of specific parental combinations for realizing the manifestation of positive heterosis for different fruit quality traits.

The strategy for breeding F_1 hybrid pumpkin is to develop parental lines through self-pollination (Sarı *et al.* 2006). However, it increases plant mean homozygosis, which is not the natural genetic state of a cross-pollinated species, and can cause inbreeding depression in mean weight and fruit length, seed production (number and weight) per fruit without affecting seed quality (weight of 100 seeds and germination) when successive generations of self-pollinations are carried out without selection (Cardoso 2004). Hazra (2006) however, advocated development of parental lines by inbred selection because no reduction in fruit, seed and fruit quality characters was evident even after four generations of successive selfings coupled with selection. Selection of divergent parents based on fruit weight, leaf area, seed weight, and fruit yield/plant per may be useful for heterosis breeding in pumpkin (Kale *et al.* 2002). Commercial hybrids were produced based on parental vine type: short × short vine and short × long vine. Marketable yield was highest in the short × long hybrids; flesh thickness however, was greatest in the open-pollinated control variety, intermediate in the short × short hybrids and lowest in the short × long hybrids (Maynard *et al.* 2001).

Commercially, hybrid seeds of pumpkin are mostly produced by: i) protection of both pistillate and staminate flowers coupled with hand pollination and ii) manual defloration with open pollination in isolated field. Preventing the development of staminate flowers in the vine of the intended female parent for extended periods by the application of growth regulators can also be employed to reduce the cost of hybrid seed production. The problem of preventing self pollination in the seed parent during hybrid seed production can also be solved with the use of male sterility; however, cytoplasmic male sterility which is utilizable for the production of hybrid seeds has not yet been found in pumpkin. Different hybrid seed production methods are discussed.

Protection of flowers and hand pollination

It is the widely employed hybrid seed production technique in pumpkin. The pistillate and staminate flowers that are to be used in manual pollination the following morning are identified in the afternoon prior to anthesis by the appearance of a slight touch of yellow at the apex of the corolla tube. Both the pistillate and staminate flowers are either covered with butter paper bags or prevented from opening by tying the tips of the corolla tube to protect the flowers from insect pollination. The following morning, pollens are transferred from the staminate flowers of the intended male parent to the stigma of the pistillate flowers of the intended female parent. In pumpkin, usually anthesis occurs in the very early morning and stigma receptivity declines sharply after 7.00 am (Hazra 2006; Mandal 2006) so the pistillate flowers of the seed parent should be pollinated with the pollen from the staminate flower of the intended male parent in the early morning hours between 5.00 to 7.00 a.m. In pumpkin crossing success ranged widely between 65.3 to 82.5%, the average being 73.9% revealing the great influence of parental genotypes on crossing success and production of hybrid seeds (Hazra 2006); the most important consideration apart from the selection of parental genotypes is hand pollination in a fully receptive stigma without injury. Fully developed hybrid seeds per fruit ranged widely between 124.3 and 356.5, mean being 256.8/fruit and an average weight of the hybrid seeds (13.47 g/100 seeds) was higher than the selfed seeds but somewhat lower than the open-pollinated seeds (Hazra 2006). Kalloo (1988) suggested two measures to improve hybrid fruit set by hybridization: i) only one or two growing shoots should be maintained and all side shoots should be pinched off and ii) only 3-4 flowers on the seed parent should be hybridized and after fruit set, the growing tips should be removed.

Removal of staminate buds and open pollination

In properly isolated hybrid seed production fields, one row of the intended male parent can be planted after every three rows of the intended female parent. Staminate flower buds are removed by hand regularly from the intended female parent and care should be taken that no staminate flowers be allowed to remain open until 2-3 fruits have set on the female parent by cross pollination, and development of later flowers may be stopped by cutting off the growing point of the vine. Bee hives are often kept in the seed production field to ensure pollination.

Chemical suppression of staminate flowers and open pollination

Monoecious sex expression of pumpkin can be regulated by exogenous application of growth regulators and ethrel, an ethylene-releasing chemical at 250 ppm, a concentration by far the most effective in increasing the number of pistillate flowers when applied at three stages of plant development i.e., first true leaf, third true leaf and fifth true leaf (Singh 2006). Plants of the intended female parent generally produce only pistillate flowers for an extended period of 25-30 days after being treated with ethrel. Further application of ethrel would not be effective so development of later staminate flowers is stopped by cutting the growing point of the vine.

Interspecific hybridization

Crossing among different *Cucurbita* species has been well documented by a number of researchers. Whitaker and Robinson (1986) from different studies on interspecific hybridization of *C. moschata*, *C. pepo*, *C. maxima* and *C. mixta* arrived at three obvious conclusions: i) F_1 hybrids can be obtained from most interspecific crossings, usually with difficulty and crossability depends on the particular accessions of the two species used as parents however, such hybrids are normally highly sterile because of impaired ability of the staminate flowers to produce functional pollen, ii) The breeding data suggest that these four species are arranged like a wheel with *C. moschata* as the hub, the others species as spokes and iii) There is no evidence for spontaneous hybridization among these four species. In all the interspecific hybrid combinations, *C. maxima* × *C. pepo* (and *vice versa*), *C. pepo* × *C. moschata*, *C. ficifolia* × *C. maxima*, (*C. maxima* × *C. moschata*) × *C. pepo*, *C. argyrosperma* × *C. moschata* (and *vice versa*), the relative nuclear DNA contents was intermediate and differed significantly from those determined in the parental species suggesting that differences in genome size within the *Cucurbita* genus are big enough to be used efficiently in determination of interspecific hybrids (Sisko *et al.* 2003). Basic objectives of interspecific crosses are to transfer one or few simply inherited desirable characters like disease resistance, good quality flesh, etc. between species. For example, good quality flesh from *C. maxima* to *C. moschata*, bushy growth habit from *C. pepo* to *C. moschata*, disease resistance from *C. lundelliana* or *C. martinezii* to *C. moschata*.

Pearson *et al.* (1951) long back could develop good quality fruits and resistance to squash bug and squash vine borer in the diploid F_1 hybrids of *C. maxima* × *C. moschata.* The F_1 hybrids were polyploidized by the use of colchicines and some of the amphidiploid lines resulting from this treatment were reasonably fertile and produced good quality fruits. Whitaker (1959) found that *C. lundellina* could be crossed with each of the cultivated *Cucurbita* species hence, served as the bridging species to transfer genes that are difficult to cross. Later, Rhodes (1959) developed an interbreeding population from crosses involving *C. lundelliana*, *C. pepo*, *C. mixta*, *C. moschata* and *C. maxima*. The transfer of single chromosome from a wild species into the genome of a cultivated species producing interspecific aneuploids has been demonstrated (Bemis 1973). In this breeding scheme, the original interspecific cross between *C. moschata* × *C. palmata* was made at the tetraploid level. The amphidiploid hybrid was backcrossed to the diploid cultivated parent, *C. moschata* and the resulting triploid was backcrossed again to *C. moschata* to produce trisomics, having 40 chromosomes of *C. moschata* and one of *C. palmata*. This procedure holds promise for transferring genes for desirable traits on one chromosome of wild species to the cultivated ones without transferring undesirable genes on other chromosomes.

Kwack and Fujieda (1985) reported that a high female line could be developed through the cross of (*C. pepo* × *C. moschata*) × *C. maxima*. The '*B*' gene, which conditions precocious yellow fruit pigmentation was successfully transferred from *C. pepo* to *C. moschata* (Paris *et al.* 1985). The interspecific hybrid obtained from the cross *C. maxima* × *C. moschata* had a high incidence of pistillate flowers and dark green colour and conical fruit shape in most interspecific hybrids were dominant to yellow colour and oblate fruit shape (Seo *et al.* 1991). New germplasm has also been developed by interspecific crosses among *C. moschata*, *C. pepo*, *C. maxima* and *C. aryrosperma*. The F_1 plants exhibited heterosis and disease resistance during vegetative growth, and showed a wide variation in botanical traits and metaxenia effects were also observed on the taste and colour of the pumpkins (Cheng *et al.* 2002). Segregates with unacceptable horticultural traits usually predominate in the F_2 generation of the interspecific crosses. The undesirable traits of the wild parental species are often dominant and consequently desirable segregates in the F_2 population may often be small. Generally backcrossing selected segregates to the cultivated species is continued until satisfactory plant types are recovered and then they are selfed until they become uniform to be used as a breeding line or new variety.

Tissue culture

Strategies based on the application of biotechnologies to crop improvement programmes generally require regeneration of whole plants from cells or tissues cultivated *in vitro*. An overview of different areas of tissue culture techniques and their applications in plant propagation and genetic improvement of *Cucurbita moschata* is presented.

Micropropagation

Shoot tip explants derived from 15 day old aseptically grown seedlings of the *C. maxima* x *C. moschata* hybrid were induced to produce multiple shoots on MS medium although, number of shoots/explant could be improved with the addition of BA (1 mg/l) and NAA (0.1 mg/l) and further with the addition of casein hydrolysate, and the regenerated shoots rooted well on half-strength MS medium supplemented with IBA and NAA at 0.1 mg/l (Islam *et al.* 1992). An efficient *in vitro* micropropagation protocol was developed for direct shoot growth using shoot tips of 5 day old explants of interspecific *Cucurbita* hybrid (Sarowar *et al.* 2003).The best condition for shoot growth was with 3 mg/l 6-benzyladenine (BA) in MS medium and the shoots of 1.0-1.5 cm length were rooted most effectively in 1.0 mg/l indole-3-butyric acid-supplemented MS medium. Rahman *et al.* (1993) could regenerate plant from internode segments of *C. maxima* × *C. moschata* hybrid on MS medium. Explanted internodal sections were induced to develop multiple shoots through direct regeneration without an intervening callus phase. The highest frequency of shoot bud formation was obtained when MS medium was supplemented with 4.4 µM benzyladenine and 0.54 µM NAA. Only one subculture of shoot bud-producing explants to the same medium was required for the development of shoots from shoot buds. Rooting of *in vitro* regenerated shoots obtained in half strength MS medium with 0.54 µM NAA.

Somatic embryogenesis

In different members of the family Cucurbitaceae, regeneration can occur either through a caulogenic or an embryogenic developmental pathway and somatic embryogenesis and plant recovery are obtained from different sources including protoplasts, but the best results are observed with explants from seedlings, especially cotyledons and hypocotyls and the genetic constitution of the mother plants seems to play a key role in the success of embryogenesis (Debeaujon and Branchard 1993). However, Kwack and Fujieda (1988) earlier reported somatic embryogenesis in cultured unfertilized ovules of *C. moschata*. Ovules excised from the ovaries at anthesis and pre-treated at 5°C for 2 days, were cultured on a 50% MS medium containing 30 g/l sucrose. The embryo sac degenerated and nucellar cells gave rise to proembryos which developed further to give embryos and few of these showed normal morphology. Upon transfer to the same medium containing 5 g sucrose and 8 g agar per litre, most embryos produced callus, but a few produced normal shoots and roots and the regenerated plants were either diploid ($2n = 40$) or tetraploid ($2n = 80$).

Embryo culture/ovule culture

Interspecific crosses among *C. moschata*, *C. pepo*, *C. maxima* and *C. aryrosperma* could be done by repeated pollination during the bud and flowering stage (Chen *et al.* 2002) although percentage fruit set and embryo size were greatly influenced by the pollen parent and/or variety (Seo *et al.* 1991). However, several researchers advocated the use of *in vitro* culture for obtaining the interspecific F_1 hybrid. The interspecific crosses *C. pepo* × *C. moschata* required embryo cultures to obtain interspecific hybrids (Munger 1990). Similarly, Seo *et al.* (1991) also advocated embryo culture technique to obtain interspecific hybrids of *C. maxima* Myako × *C. moschata* Butter Bush, *C. moschata* PM142 × *C. maxima* Myako. Earlier, immature embryos of *C. maxima* × *C. pepo* and *C. pepo* × *C. moschata* hybrids were successfully regenerated in growth medium (Rakoczy-Trojanowska and Malepszy 1986; Kwack and Fujieda 1987). Recently, F_1 hybrid plants could be obtained from the crosses: *C. maxima* × *C. pepo* (and *vice versa*), *C. pepo* × *C. moschata*, *C. ficifolia* × *C. maxima*, (*C. maxima* × *C. moschata*) × *C. pepo*, *C. argyrosperma* × *C. moschata* using embryo rescue technique (Sisko *et al.* 2003). Embryo germination was favourable on MS medium containing 6% sucrose, and successful multiple shoots could be obtained by reculturing shoot tips from developing seedlings in MS medium supplemented with 0.5-1.0 mg/l benzyladenine (BA). Transplantable plantlets were obtained when shoot tips were cultured on the MS media containing 1.0 mg/l BA and 0.5 mg/l IBA or 0.5-3.0 mg/l IAA in combination (Seo *et al.* 1991). Interspecific hybrids could also be obtained through ovule culture. Embryos of four interspecific combinations between *C. moschata* (cultivars Seoulmadi and Puthobag) and *C. pepo* (cultivar Ford Zucchini) did not germinate in intact ovules when plated on artificial media but those in cut ovules germinated well. Embryo germination rate was higher at 27 than at 23°C, and medium containing half-strength MS salts supplemented with 35 g/l sucrose gave the best results (Hong *et al.* 1994).

Protoplast fusion

The idea of protoplast fusion lay on overcoming natural inherent barrier in producing sexual crosses between closely related as well as unrelated species for development of utilizable hybrids, and few such reports involving *C. moschata* and other species of Cucurbitaceae can be cited. Yamaguchi and Shiga (1993) reported the characteristics of the regenerated plants from the fused protoplasts of melon (*Cucumis melo*) and pumpkin (interspecific hybrid, *C. maxima* × *C. moschata*). Protoplasts of melon and pumpkin were fused by electrofusion. Leaves from the 188 regerants obtained from calluses were analysed for electrophoretic banding pattern of acid phosphatase, 33 putative somatic hybrids could be selected and of these 3 were grown in the greenhouse. These plants possessed dark green leaves and leaf shape varied from round to deeply serrated. Electrophoregrams exhibited both parents' banding patterns. Chromosome numbers were 2n = 24-28 in one root tip. As the plants developed, they began to closely resemble melons in traits of leaves and other plant parts. At the flowering stage, chromosome numbers of root tips from cuttings of axillary buds were almost all 2n = 24. The acid phosphatase band from pumpkin was not found in the isoenzyme pattern. Both fertility and germination rate of the pollens were high and fruits and seeds were obtained by self pollination. The traits and taste of the fruits were almost the same as for melon. It appeared that at the early growth stages, regenerated plants showed hybridity owing to the introduction of a part of the pumpkin chromosome, but the hybridity disappeared at later growth stages which revealed the difficulty of transferring genes between crops belonging to different genera of the Cucurbitaceae. Polyethylene glycol mediated protoplast fusion between *Cucumis sativus* and *Cucurbita moschata* and *Cucumis sativus* and *Cucurbita ficifolia* has also been reported (Zhang and Liu 1998). Division of protoplasts isolated from the cotyledons of cucumber (*Cucumis sativus*) cultivars Erzaozi and Mici was inhibited when treated with 10 mg/l rhodamine 6G (R6G) for 15 min. Cotyledonary protoplasts of China squash (*Cucurbita moschata*) and Malabar gourd (*Cucurbita ficifolia*) were also inhibited after treatment with 1 and 1.3 mmol IAA per litre, respectively, for 10 min. Fused protoplasts resumed division when treated with 25-35% polyethylene glycol (6000) for 5-10 min. Calli were obtained from the fusion combinations of *C. sativus* with *C. moschata* and *C. ficifolia* and somatic hybrids could be identified.

RESISTANCE BREEDING

Resistance to diseases

Disease resistance has become the major breeding objective in breeding programmes of pumpkin and other *Cucurbita* species. Pumpkin is particularly susceptible to different fungal diseases particularly powdery mildew (*Sphaerotheca fulliginea*), downy mildew (*Pseudoperonospora cubensis*) and root rot (*Phytopthora capsici*) and several virus diseases viz., watermelon mosaic virus, cucumber mosaic virus, squash mosaic virus, bottle gourd mosaic virus, cucurbit latent virus, squash leaf curl virus, zucchini yellow mosaic, papaya ring spot virus, pumpkin mosaic virus, etc. Pumpkin yellow vein mosaic (PYVMV) disease symptoms occur frequently in pumpkins in India. Diseased plants show vein yellowing, which sometimes coalesces to form chlorotic patches and infected plants are stunted with premature dropping of flowers and PYVMV is most similar to the tomato leaf curl New Delhi virus from India and appears to be a new strain of this virus (Muniyappa *et al.* 2003). It was also reported later that the begomovirus associated diseases like, Tomato leaf curl New Delhi virus-*Luffa* in cucurbits are emerging problems in India (Sohrab *et al.* 2006). In general cucurbit volunteer plants and weeds act as important volunteer plants of different viruses. Population of the worlds's most economically important biotypes of *Bemisia tabaci*, namely "B" and "Q" is particularly significant in the spread of geminivirus with devastating effects to crop production (Sseruwagi *et al.* 2005).

Some cultivars or accessions of pumpkin showed different levels of field resistance to some diseases like powdery mildew (Sowell and Coreley 1973, Kabitarani and Bhagirath 1991; Wessel-Beaver 1993), downy mildew (Wessel-Beaver 1993), root rot (Kuginuki *et al.* 1994) squash virus (Salama and Sill Jr. 1968), squash leaf curl virus (McCreight 1984) and watermelon virus (Maluf *et al.* 1986). However, in most cases the level of resistance to the diseases found in the cultivars of *C. moschata* is less than desirable. Hence, breeding for disease resistance stands heavily on the germplasm developed by interspecific hybridization involving a group of wild *Cucurbita* species which are closely allied to the cultivated *Cucurbita* and to some extent cross compatible with the cultivars.

Feral species of *Cucurbita* are found resistant to many diseases viz., *C. lundelliana* and *C. martinezii* are resistant to powdery mildew (Rhodes 1964; Contin and Munger 1977) and different viruses such as watermelon mosaic, cucumber masaic virus and Zucchini yellow mosaic virus (Provvidenti *et al.* 1978; Maluf *et al.* 1986; Robinson *et al.* 1988), *C. equadorensis* is resistant to papaya ringspot virus (Herrington *et al.* 1989) and *C. andreana*, *C. lundelliana*, *C. equadorensis* and *C. foetidissima* are resistant to bacterial wilt caused by *Erwinia trachiiphila* (Watterson *et al.* 1971). The inheritance of resistance to different diseases in *Cucurbita* species is reviewed in **Table 4**. Generally backcross breeding method is employed for the transfer of resistance from the donor parent to the commercial cultivar (Munger and Washek 1983; Pink 1987).

Table 4 Inheritance of resistance to different diseases in *Cucurbita* species.

Resistance to the disease	**Inheritance**	**Reference**
Powdery mildew in *C. lundellina*	Single dominant gene	Rhodes 1964
Powdery mildew in *C. martinezii*	A major gene showing partial dominance and influence of a modifier	Contin and Munger 1977
	A single dominant gene with some modifications by minor genes	Cho *et al.* 2004
Papaya ring spot virus in *C. ecuadorensis*	Polygenic inheritance with additive gene action	Herrington *et al.* 1989
Zucchini yellow mosaic virus in *C. ecuadorensis*	Polygenic inheritance with additive gene action	Paran *et al.* 1989
Watermelon mosaic virus in *C. maxima*	Additive gene action	Maluf *et al.* 1985
Watermelon mosaic virus and Zucchini yellow mosaic virus in *C. moschata*	The same dominant gene "*Zym*" or two closely linked genes	Gilbert *et al.* 1993
Cucumber mosaic virus in *C. pepo*	Two unlinked recessive genes	Pink 1987
Zucchini yellow mosaic virus in *C. moschata*	Single dominant gene "*Zym*"	Paris *et al.* 1988; Robinson *et al.* 1988; Brown *et al.* 2003
Watermelon mosaic virus in *C. moschata*	Single dominant gene "*Wmv*"	Brown *et al.* 2003
Cucumber mosaic virus in *C. moschata*	Single dominant gene "*Cmv*"	Brown *et al.* 2003
Papaya ringspot virus W in *C. moschata*	Single recessive gene "*prv*"	Brown *et al.* 2003
Pumpkin mosaic in *C. moschata*	Single recessive gene	Latha and Gopalakrishnan 1993
Yellow vein mosaic in *C. moschata*	Single dominant gene	Latha and Gopalakrishnan 1993
Whitefly (*Bemisia argentifolii*) induced silverleaf disorder in in *C. moschata*	Single dominant gene	Gonzalez and Wessel-Beaver 2002

Fungal disease

The response of *C. moschata* varieties to three isolates of downy mildew causing fungus *Pseudoperonospora cubensis* obtained from *Cucumis sativus* was investigated and *C. moschata* was highly susceptible to all the three isolates of the pathogen (Lebeda and Kristova 1993) however, in Puerto Rico, Wessel Beaver (1993) could identify 45 accessions as field resistant to this fungus which were all from Central America mainly Mexico.

Ma *et al.* (2002) have used aerial transmission of conidiophores of *Sphaerotheca fulliginea* from the infected plants at four-true leaf stage to inoculate pumpkin and other cucurbit leaves. The time course progress and cytohistology of infection of susceptible and resistant genotypes of *C. moschata* by conidia of *Sphaerotheca fulliginea* causing powdery mildew were investigated. In the susceptible genotype only 4% of the conidia germinated at 5 hour post inoculation period (hpi), invasion of the host cell began at 15 hpi with the formation of haustorium, a non-appressorial germ tube formed at 18 hpi, secondary hyphae developed at 2 days post inoculation (dpi) and conidiophore formation began at 3 dpi, maturing at 4 dpi and in the resistant genotypes, conidial germination, invasion of the host cell and formation of non-appressorial germ tubes progressed similarly but growth of the secondary hyphae, conidiophore formation and sporulation were highly inhibited (Kabitarani *et al.* 1997). However, none of the germplasm was immune excepting the isolate P 14 from Mizoram, India which had the highest degree of resistance (Kabitarani and Bhagirath 1991a) and of the three types of resistance displayed by the *C. moschata* germplasm: to spore germination, penetration and sporulation, suppression of sporulation showed the highest genotype specificity and is considered to be the most useful in powdery mildew resistance breeding programme (Kabitarani and Bhagirath 1991b). Ma *et al.* (2002) also observed that the papilla formation after inoculation of the fungus was not associated with resistance but haustoria formation under papilla marked successful penetration. Resistance to powdery mildew in *C. lundellina* is governed by single dominant gene and can be transferred to the cultivars of *C. moschata* (Rhodes 1964). Powdery mildew resistance of *C. martinezii*, controlled by a single dominant gene with some modifications by minor gene was successfully transferred to the *C. moschata* cultivar "Wonye 402" through interspecific hybridization (Cho *et al.* 2004c). Two powdery mildew oriental squash (*C. moschata*) cultivars "Mansu" and "Chensu"were developed at Korea through interspecific hybridization between *C. moschata* local cultivar "Jecheonjaerae" × *C. martinezii* followed by selection of powdery mildew resistant and non-bitter genotype from BC_2F_1 population which was selfed twice then backcrossed to the *C. moschata* local cultivar "Jecheonjaerae" and finally selfed thrice (Cho *et al.* 2004a, 2004b).

Kuginuki *et al.* (1994) studied different methods of inoculating *Phytopthora capsici* causing root rot and found that soil drenching inoculation using zoospores in suspendsion was more effective than spray and needle inoculation. It was further concluded that 15 or 22 day old seedlings are suitable for screening the genotypes of *C. moschata* and other cucurbits for resistance to root rot using inoculum concentrations of above 1×10^3/ml. Henz and Lima (1998) advocated inoculation at the cotyledonary stage, 10 days after sowing by placing 3 ml of inoculum (104 zoospores/ ml) at the base of the seedling. All the *C. moschata* cultivars were susceptible in one screening trial (Henz and Lima 1998) while the other trial revealed that *C. moschata* varieties Shimakabocha, Shishigatani and Jiki were highly resistant to root rot (Kuginuki *et al.* 1994).

Virus disease

In the screening of different genotypes for virus resistance, Provvidenti *et al.* (1978) advocated inoculation of the seedlings of *Cucurbita* species by dusting carborundum abrasive on young leaves and rubbing them with inoculum prepared by triturating infected squash or cucumber leaves with 0.05 M phosphate buffer at pH 7.0 and resistance was evaluated on the basis of symptoms and by the ability of the plant to avoid systemic infection, as indicated by recovery tests in which extracts from the plants were used to inoculate susceptible squash or cucumber plants. Viruses in the shoot samples can be tested by different biotechnological techniques like, enzyme linked immunosorbent assay (ELISA) using antibodies to specific virus, tissue blot immunosorbent assay (TBIA) using generic luteovirus antibodies (Coutts and Jones 2005) and nucleic acids hybridization test using radiolabelled probe to putative coat protein gene of the virus (Sohrab *et al.* 2006).

Zucchini yellow mosaic potyvirus (ZYMV), the most studied virus of cucurbits is prevalent in different cucurbit growing agro-ecosystems in the world and all isolates are transmitted in a non-persistent manner by *Aphis gossypii* and *Myzus persicae* (Mahgoub *et al.* 1998). A full length cDNA clone of the RNA genome of the ZYMV aws constructed downstream from a bacteriophage T 7 RNA polymerase promoter, a single extra guanosine residue not present in ZYMV RNA was added to the 5′ and 3′ ends. Capped (m7GppG) ZYMV RNA transcripts were infectious in 10 of 91 *C. pepo* test plants and uncapped RNA transcripts were not infectious (Gal-On *et al.* 1991). The 3′ terminal sequences of the ZYMV genome from Beijing, China was characterized by RT-PCR from the total RNA of infected leaves and cloned into the pMD18-T vector, and the sequences were 1269 bp long and contained a coat protein (CP) gene, which consisted of 837 nucleotides encoding 279 amino acids (Zhang *et al.* 2006). Different accessions of *C. moschata* viz., Minina, originating from Portugal (Gilbert *et al.* 1993), Nigerian Local (Provvidenti *et al.* 1997; Brown *et al.* 2003), BGH 1934, BGH 1937 and BGH 1943 (Moura *et al.* 2005) and wild species *C. ecuadorensis* (Paran *et al.* 1989; Provvidenti *et al.* 1997) are known to carry resistance against this virus. Transfer of ZYMV resistance from the resistant *C. moschata* cultivars and *C. ecuadorensis* to the varieties of *C. moschata*, *C. pepo* and *C. maxima* was successful in some cases resulting in the development of new cultivars showing high level of resistance (Provvidenti *et al.* 1997; Desbiez *et al.* 2003). No RAPD markers linked to ZYMV resistance were found (Brown *et al.* 2003).

Papaya ringspot poty virus (PRSV) contains 2 major groups, type P which infects Chenopodiaceae, Cucurbitaceae and Cariceae (PRSV P) and type W (PRSV W) which infects Chenopodiaceae and Cucurbitaceae (Wang and Yeh 1998). Resistance to PRSV W in *C. ecuadorensis* appeared to be controlled more consistently by a polygenic system with additive gene effects being predominant and no strong association existed between PRSV W resistance and undesirable economic characters (Herrington *et al.* 1989). These findings amply supports the idea of developing PRSV W resistant *C. moschata* cultivars from the interspecific cross *C. moschata* × *C. ecuadorensis* following backcross or backcross-pedigree method of breeding. Engineered mild strain of papaya ringspot virus for broader cross protection in cucurbits has been studied. Papaya ringspot virus (PRSV) HA5-1, a mild mutant of type P Hawaii severe strain (PRSV P-HA), has been widely used for the control of PRSV type P strains in papaya, but did not provide practical protection against PRSV type W strain in cucurbits. In order to widen the protection effectiveness against W strains, chimeric mild strains were constructed from HA5-1 to carry the heterologous 3′ genomic region of a type W strain W-CI. The recombinant carrying both the heterologous coat protein (CP) coding region and the 3′ untranslated region (3′UTR), but not the heterologous CP coding region alone which significantly enhanced the protecttion against W-CI. These recombinants provide broader protection against both type W and P strains in cucurbits (You *et al.* 2005).

Two species *C. ecuadorensis* and *C. foetidissima* resistant to watermelon mosaic virus 1 (WMV 1) and watermelon mosaic virus 2 (WMV 2) as well as cucumber mosaic virus (CMV) can be used to develop virus resistant *C. moschata* cultivars following interspecific hybridization (Provvidenti *et al.* 1978). Resistance to WMV 2 potyvirus was also found in a *C. moschata* cultivar "Menina" which is also resistant to zucchini yellow mosaic potyvirus and resistance is manifested through limiting or blocking the migration of WMV 2 from the inoculated leaves or cotyledons (Gilbert *et al.* 1993).

Multiple disease resistance

An excellent method of combining sources of disease resistance in *Cucuurbita* species was described by Rhodes (1964). This method consisted of an interbreeding population or gene pool: *C. pepo*, *C. mixata*, *C. maxima* and *C. moschata*. Plants from this gene pool are used in a series of bridging crosses using *C. lundelliana* as a bridge species to transfer specific genes between incompatible species. By utilizing this method bush habit from *C. pepo* to *C. moschata* and powdery mildew resistance to *C. moschata* from *C. lundelliana* was transferred (Rhodes 1964). From this gene pool, multiple resistances to powdery mildew, downy mildew and squash mosaic virus as obtained in summer squash, *C. pepo* (Sitterly 1972) can also be developed in *C. moschata* cultivars. Several multiple virus resistant cucurbit crops particularly, muskmelon, cucumber, squash and watermelon are already available (Gaba *et al.* 2004) and due research attention is needed to develop multiple disease resistant pumpkin (*C. moschata*) through biotechnology.

Resistance to insect pests

Breeding for insect resistance is difficult because of the intricate difficulties of three phases of work: i) rearing the insects or obtaining natural infestation ii) exposing test plants at the appropriate stage to a uniform number of insects and iii) accurately assaying the response (Whitaker and Robinson 1986). Very limited work has been done so far on breeding *C. moschata* cultivars resistant to insect pests. Few reports on the identification of source of resistance in pumpkin include combined resistance to prickle worm, striped cucumber beetle, and serpentine leaf miner (Hall and Painter 1968), resistance to red pumpkin beetle (Grewal 1982) and resistance to leaf silvering disorder in response to feeding by *Bemisia argentifolii* at immature stage (Wessel-Beaver and Katzir 2000). Dhillon and Sharma (1989) suggested that cage evaluation may be used in screening for resistance to red pumpkin beetle. It has been found that *C. moschata* cultivars resistant to leaf silvering disorder had non-mottled leaves indicating a possible relationship between the genetically controlled silver leaf mottling and expression of the silver leaf disorder due to feeding of *Bemisia argentifolii* (Wessel-Beaver and Katzir 2000).

CONCLUSIONS AND FUTURE PERSPECTIVES

Of the three main cultivated species of *Cucurbita*, pumpkin or tropical pumpkin *C. moschata* is the least studied species although it a highly polymorphic domesticate and most widely cultivated *Cucurbita* in the tropics. Little is known about genetics of different qualitative and quantitative characters of *C. moschata* so more genetic information for important characters including plant habit, sex expression, fruit set, fruit shape, fruit size, β-carotene and sugar contents of flesh and disease resistance will help framing the breeding strategy. The major objectives in breeding pumpkin have been to improve fruit yield and quality of fruit flesh. Although both bush habit and precocious yellow fruit colour from *C. pepo* has been successfully transferred to *C. moschata*, development of semi-viny pumpkin cultivars with high vitamin A content need some more focus. The primary breeding method, particularly in most of the tropical countries has been inbred selections and selections from intraspecific crosses. It has been well documented that different fruit characters and yield including β-carotene and sugar contents of flesh and even resistance to diseases improves by inbred selection. The F_1 hybrids have been shown to be more desirable than open pollinated cultivars but development of less viny hybrids with the involvement of breeding lines emanated from *C. pepo* × *C. moschata* crosses warrants much attention. Development of either gynoecious line or cytoplasmic male sterile line in pumpkin will improve commercial production of hybrid seeds. Interspecific crosses among different *Cucurbita* species, different tissue culture techniques to develop the hybrids and breeding methods to utilize the desirable genes from other *Cucurbita* species have been well understood and documented. Pumpkin breeding involving both cultivable (*C. pepo*, *C. maxima*) and closely allied wild species particularly *C. lundelliana*, *C. ecuadorensis* and *C. martinezii* with proper biotechnological interventions to develop improved cultivars with multiple disease resistance should be the major goal of pumpkin breeding programme.

ACKNOWLEDGEMENTS

P. Hazra acknowledges the financial support rendered by the Department of Agriculture, Government of West Bengal, India through sanctioning the research project "Exploration and utilization of bio-diversity for development of some vegetable crops in West Bengal" which helped to study different aspects of breeding pumpkin.

REFERENCES

Adeoye AA, Coyne DP (1981) Inheritance of resistance to trifluralin toxicity in *Cucurbita moschata* Poir. *HortScience* **16**, 774-775

Amaral-Junior AT, Casali VWD, Cruz CD, Silva DJH, Silva LFC, da Silva DJH, da Silva LFC (1999) Estimates of phenotypic, genotypic and environmental correlations between seven morphological and agronomic characters in eight pumpkin accessions. *Bragantia* **53**, 163-166

Andres TC (2004) Diversity in tropical pumpkin (*Cucurbita moschata*): a review of intraspecific classifications. *Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004*, 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech Republic, 12-17 July, 2004, pp 107-112

Bemis WP (1973) Interspecific aneuploidy in *Cucurbita*. *Genetic Research* **21**, 221-228

Bindu S, Mahakal KG, Kale PB, Sakahre SB, Chitra KR (2000) Genetic variability in pumpkin (*Cucurbita moschata* Duch. ex. Poir.). *Annals of Plant Physiology* **14**, 66-68

Brown RN, Bolanos HA, Myers JR, Jahn MM (2003) Inheritance of resistance to four cucurbit viruses in *Cucurbita moschata*. *Euphytica* **129**, 253-258

Brown RN, Myers JR (2001) RAPD linked to morphological and disease resis-tance traitd in squash. *Report of the Cucurbit Genetics Cooperative* **24**, 91-93

Cao JS, Yu HF, Ye WZ, Yu XL, Liu LC, Wang YQ, Xiang X (2005) Identification and characterisation of a gibberellin-related dwarf mutant in pumpkin (*Cucurbita moschata*). *Journal of Horticultural Science and Biotechnology* **80**, 29-31

Cardoso AII (2004) Depression by inbreeding after four successive self-pollination squash generation. *Scientia Agricola* **61**, 224-227

Cardoso AII, Silva N, Della-Vecchia PT (1995) Inheritance of immature fruit colour in *Cucurbita moschata*. *Horticultura Brasileira* **13**, 170-171

Carle RB, Maynard DN, Wessel-Beaver L, Katzir N, Paris HS (2000) Tropical pumpkin hybrid development: landraces to hybrid cultivars. *Acta Horticulturae* **510**, 95-100

Chakravarty HL (1982) *Fascicles of Flora of India Fascicle 11, Cucurbitaceae*, Botanical Survey of India, Howrah, India, 136 pp

Chattopadhyay P (1984) Inheritance of some morphological attributes and yield components of *C. moschata* Poir. PhDThesis, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India

Chaturvedi S (2001) Qualitative traits of some pumpkin. *Vegetable Science* **28**, 182-183

Cheng YA, Zhang BK, Zhang EH, Zhao ZL (2002) Germplasm innovation by interspecific crosses in pumpkin. *Report of the Cucurbit Genetics Cooperative* **25**, 56-57

Cho MC, Heo YC, Kim JS, Om YH, Mok IG, Hong KH, Park HG (2004a) A new oriental sqash (*Cucurbita moschata*) cultivar, "Mansu" resistant to powdery mildew. *Korean Journal of Breeding* **36**, 111-112

Cho MC, Heo YC, Kim JS, Om YH, Mok IG, Hong KH, Park HG (2004b) A new oriental squash (*Cucurbita moschata*) cultivar, "Chensu" resistant to powdery mildew. *Korean Journal of Breeding* **36**, 113-114

Cho MC, Om YH, Heo YC, Kim JS, Park HG (2004c) Inheritance of powdery mildew resistance, bitterness, fruit rind hardness and fruit shape in *Cucurbita* spp. *Korean Journal of Breeding* **36**, 271-275

Choer E (1999) Morphological evaluation of *Cucurbita* spp. accessions. *Agropecuaria Clima de Temperado* **2**, 151-158

Chung HD, Youn SJ (1998) Chemical composition and quality evaluation of ripe fruit of the Korean native squash (*Cucurbita moschata*). *Journal of the Korean Society for Horticultural Science* **39**, 510-516

Contin M, Munger HM (1977) Inheritance of powdery mildew resistance in interspecific crosses with *Cucurbita martinezii*. *HortScience* **12**, 397

Coutts BA, Jones RAC (2005) Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. *Australian Journal of Agricultural Research* **56**, 847-858

Damarany AM (1989) Improvement of pumpkin by inbreeding and visual selection. *Assuit Journal of Agricultural Science* **20**, 173-183

Daniel AL, Brecht JK, Sims CA, Maynard DN (1996) Sensory analysis of bush and vining types of tropical pumpkin. *Proceedings of the Florida State Horticultural Society* **108**, 312-316

Danilcenko H, Genyte J (1999) Biochemical Research on pumpkin varieties. *Sodininkyste ir Darzininkyste* **18**, 62-69

Debeaujon I, Branchard M (1993) Somatic embryogenesis in Cucurbitaceae. *Plant Cell, Tissue and Organ Culture* **34**, 91-100

Decker-Walters DS, Walters TW, Poslusny U, Kevan PG (1990) Genealogy and gene flow among annual domesticated species of *Cucurbita*. *Canadian Journal of Botany* **68**, 782-789

Della-Vecchia PT, Terenciano SP, Terenciano A (1993) Breeding bush types of *C. moschata* with field resistance to PRSV-W. *Report of the Cucurbit Genetics Cooperative* **16**, 70-72

Denna DW, Munger HM (1963) Morphology of the bush and vine habits and the allelism of the bush genes in *Cucurbita maxima* and *C. pepo* squash. *Proceedings of the American Society for Horticultural Science* **82**, 370-377

Desbiez C, Gal-On A, Girard M, Wipf-Scheibel C, Lecoq H (2003) Increase in zucchini yellow mosaic virus symptom severity in tolerant zucchini cultivars is related to a point mutation in P3 protein and is associated with a loss of relative fitness in susceptible plants. *Phytopathology* **93**, 1478-1484

Dhillon NPS, Sharma BR (1989) Relationship between field and Cage assessments for resistance to red pumpkin beetle in summer squash. *Euphytica* **40**, 63-65

Doijode SD (1983) Heterotic performance for TSS and carotene in pumpkin (*C. moschata*). *Genetica Agraria* **37**, 233-238

Doijode SD, Nath P, Sulladmath UV (1982) Hybrid vigour in pumpkin (*C. moschata*). *Genetica Agraria* **36**, 87-94

Doijode SD, Sulladmath UV (1986) Genetic variability and correlation studies in pumpkin (*C. moschata*). *Mysore Journal of Agricultural Science* **20**, 59-61

Doijode SD, Sulladmath UV (1988) Genetics of certain vegetative and flowering characters in pumpkin (*C. moschata*). *Agricultural Science Digest, India* **8**, 203-206

Doijode SD, Sulladmath UV, Kulkarnio RS (1987) Inheritance of certain seed characters in pumpkin (*C. moschata*) *Vegetable Science* **14**, 143-146

Ferriol M, Pico B, de Cordova PF, Nuez F, de Cordova PF (2004) Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. *Crop Science* **44**, 653-664

Ferriol M, Pico MB, Nuez F (2003) Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. *Genetic Resources and Crop Evolution* **50**, 227-238

Gaba V, Zelcer A, Gal-On A (2004) Cucurbit biotechnology – the importance of virus resistance. *In Vitro Cellular and Developmental Biology – Plant* **40**, 346-358

Galka Al (1987) Inheritance of some economically useful traits in first generation hybrids of *Cucurbita*. *Oveshchevedstvo I Bakhchevodstvo* **32**, 45-46

Gal-On A, Antignus Y, Rosner A, Raccah B (1991) Infectious *in vitro* RNA transcripts derived from cloned cDNA of the cucurbit potyvirus, zucchini yellow mosaic virus. *Journal of General Virology* **72**, 2639-2643

Gilbert AF, Lecoq H, Pitrat M, Nicolet JL (1993) Resistance of *Cucurbita moschata* to watermelon mosaic virus type 2 and its genetic relation to resistance to zucchini yellow mosaic virus. *Euphytica* **69**, 231-237

Goldberg RB, Bemis WP, Siegel A (1972) Nucleic acid hybridization studies within the genus *Cucurbita*. *Genetics* **72**, 253-266

Gonzalez RM, Wessel-Beaver L (2002) Resistance to silverleaf disorder is controlled by a single recessive gene in *Cucurbita moschata* Duchesne. *Report of the Cucurbit Genetics Cooperative* **25**, 49-50

Grewal SS (1982) Studies on resistance to cucurbits to red pumpkin beetle, *Aulacophora foveicollis*. *Thesis Abstract* **8**, 365

Gupta PN, Rai M (1990) Genetic diversity in cucurbits – pumpkin. *Indian Horticulture* **36**, 1

Gwanama C, Botha AM, Labuschagne MT (2001) Genetic effects and heterosis of flowering and fruit characteristics of tropical pumpkin. *Plant Breeding* **120**, 271-272

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

Gwanama C, Mwala MS, Nichterlein K (1998) Path analysis of fruit yield components of *Cucurbita moschata* Duch. *Tropical Agricultural Research and Extension* **1**, 19-22

Gwanama C, Nichterlein K, Lungu D, Simabwachi W (2002) Variation of fruit β-carotene content of tropical pumpkin (*Cucurbita moschata* Duch.ex. Poir) landraces in Zambia. *Plant Genetic Resources Newsletter* **129**, 44-46

Haber ES (1928) Inbreeding in the table Queen (Des Moines) squash. *Proceedings of the American Society for Horticultural Science* **25**, 111-114

Hall CV, Painter RH (1968) Insect resistance in *Cucurbita*. *Kansas Experimental Station Technical Bulletin*, 256 pp

Herrington ME, Byth DE, Teakle DS, Brown PJ (1989) Inheritance of resistance to papaya ringspot virus type W in hybrids between *C. ecuadorensis* and *C. maxima*. *Australian Journal of Experimental Agriculture* **29**, 253-259

Hazra P (2006) Seed production technology in pumpkin. In: Ram HH, Singh HP (Eds) *Cucurbit Breeding and Production Technology*, Proceedings of the National Seminar on Cucurbits, College of Agriculture, Pantnagar, Uttaranchal, India, pp 183-194

Hazra P, Banerjee MK (2005) *Varieties in Vegetable crops in India*, Kalyani Publishers, Ludhiana, pp 102-134

Henz GP, Lima MF (1998) Plantlet resistance of cucurbit cultivars to root rot caused by *Phytophthora capsici*. *Pesquisa Agropecuaria Brasileira* **33**, 853-859

Hong KH, Om YH, Park HG (1994) Interspecific hybridization between *Cucurbita pepo* and *C. moschata* through ovule culture. *Journal of the Korean*

Society for Horticultural Science **35**, 438-448

Hong KH, Om YH, Heo YC, Ko KD (1997) Development of semi-bush type "Aehobag" squash (*Cucurbita moschata*) lines, "Wonye 401" and "Wonye 402". *RDA Journal of Horticultural Science* **39**, 132-134

Hong KH, Om YH, Heo YC, Ko KD (1998) Development of semi-bush type "Puthobag" squash (*Cucurbita moschata*) lines, "Wonye 403" and "Wonye 404". *RDA Journal of Horticultural Science* **40**, 166-168

Hurd PD, Linsley EG, Whitaker TW (1971) Squash and gourd bees and the origin of the cultivated *Cucurbita. Evolution* **25**, 218-234

Islam AKMR, Joarder OI, Rahman SM, Hossain M (1992) Micropropagation in *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch. through seedling derived shoot tips. *Indian Journal of Horticulture* **49**, 249-252

Jeon HJ, Been CG, Hong KH, Om YH, Kim BD (1994) Identification of Cucurbitaceae cultivars by using RAPD markers. *Journal of the Korean Society for Horticultural Science* **35**, 449-456

Kabitarani A, Bhagirath T (1991a) Sources of powdery mildew resistance in *Cucurbita moschata. Indian Phytopathology* **44**, 260-261

Kabitarani A, Bhagirath T (1991b) Resistance of squash (*Cucurbita moschata*) to infection process and sporulation of powdery mildew fungus (*Sphaerotheca fuliginea*). *Indian Journal of Agricultural Sciences* **61**, 783-784

Kabitarani A, Bhagirath T, Singh NI (1997) Cytohistology of interaction between *Cucurbita moschata* and *Sphaerotheca fuliginea* - conidial infection. *Journal of Mycology and Plant Pathology* **27**, 55-59

Kale VS, Patil BR, Bindu S, Paithankar DH (2002) Genetic divergence in pumpkin (*Cucurbita moschata*). *Journal of Soils and Crops* **12**, 213-216

Kalloo G (1988) *Vegetable Breeding* (Vols I and II), CRC Press, Florida, pp 213, 237

Kirkpatrick KJ, Decker DS, Wilson HW (1985) Allozyme differentiation in the *C. pepo* complex: *C. pepo* var. *medullosa* vs. *C. texana. Economic Botany* **39**, 289-299

Kirkpatrick KJ, Wilson HD (1988) Interspecific geneflow in *Cucurbita: C. texana* vs. *C. pepo. American Journal of Botany* **75**, 519-527

Koutsika-Sotiriou M, Traka-Mavrona E, Tsivelikas AL, Mpardas G, Mpeis A, Klonari E (2004) Use of genetic resources in a dual approach toward selecting improved scion/rootstock grafting combinations of melon (*Cucumis melo*) on *Cucurbita* species. *Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004*, 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech-Republic, 12-17 July, 2004, pp 163-167

Kubicki B, Walczak B (1976) Variation and heritability of β-carotene content in some cultivars of *Cucurbita* species. *Genetica Polonica* **17**, 531-544

Kuginuki Y, Kawaide T, Kanno T (1994) Screening methods for varietal differences in resistance to *Phytophthora capsici* Leonian in *Cucurbita. Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea, Series A: Vegetables and Ornamental Plants* **9**, 1-11

Kwack SN, Fujieda K (1985) Breeding high female lines through interspecific hybridization of *Cucurbita. Report of the Cucurbit Genetics Cooperative* **8**, 78-79

Kwack SN, Fujieda K (1987) Seed abortion and techniques for obtaining hybrids in interspecific crosses of *Cucurbita. Journal of the Japanese Society for Horticultural Science* **55**, 455-460

Kwack SN, Fujieda K (1988) Growth habit in populations of an interspecific cross *C. pepo* × *C. moschata. Journal of the Korean Society for Horticultural Science* **29**, 9-12

Kwack SN, Fujieda K (1988) Somatic embryogenesis in cultured unfertilized ovules of *Cucurbita moschata*. *Journal of the Japanese Society for Horticultural Science* **57**, 34-42

Kumaran SS, Natarajan S, Thamburaj S (1998) Correlation and path analysis studies in pumpkin (*Cucurbita moschata* Poir.). *South Indian Horticulture* **46**, 138-142

Lakshmi LM, Haribabu K, Reddy GLK (2003) Genetic divergence in pumpkin. *Indian Journal of Horticulture* **60**, 363-367

Latha P, Gopalakrishnan TR (1993) Inheritance of mosaic diseases and silvery leaf trait in pumpkin (*Cucurbita moschata* Poir). *Horticultural Journal* **6**, 97-101

Lebeda A, Kristkova E (1993) Resistance of *Cucurbita pepo* and *Cucurbita moschata* varieties to cucurbit downy mildew (*Pseudoperonospora cubensis*). *Plant Varieties and Seeds* **6**, 109-114

Lee YH, Jeon HJ, Hong KH, Kim BD (1995) Use of random amplified polymorphic DNAs for linkage group analysis in interspecific hybrid F_2 segregation of *Cucurbita. Journal of the Korean Society for Horticultural Science* **36**, 323-330

Liu XJ, Li YJ, Zhao Y, Fang C, Sun ZH, Wang ML (2004) Analysis of genetic variation in some Chinese cultivated pumpkin and squash resources by random amplified polymorphic DNA (RAPD) markers. *Southwest China Journal of Agricultural Sciences* **17**, 567-571

Lozanov P (1969) Heterosis in some intervarietal pumpkin hybrids. *Genetika I Selekeija Sofia* **2**, 277-278

Ma Q, Cui HW, Qiang L, Sun H (2002) Resistance of cucurbits to the powdery mildew, *Sphaerotheca fuliginea* (Schlecht.) Poll. *Report of the Cucurbit Genetics Cooperative* **25**, 63-64

Mahajan VH, Sirohi PS (2000) Heterosis in pumpkin (*Cucurbita moschata* Duch. ex. Poir). *Punjab Rao Krishi Vidyapeeth Research Journal* **26**, 36-39

Mahgoub HA, Desvíes C, Wipf-Scheibel C, Dafalla G, Lecoq H (1998) Biological and selological variability of zucchini yellow mosaic virus in Sudan. *Journal of Phytopathology* **146**, 333-337

Maluf WR, Moura WDEM, Silva ISDA, Castelo-Branco M (1986) Screening of *Cucurbita* species accessions for resistance to watermelon mosaic virus-1. *Revista Brasileira de Genética* **9**, 161-167

Maluf WR, Silva ISDA, Moura WDEM (1985) Inheritance of Watermelon mosaic virus 1 (WMV-1) resistance in squash (*C. maxima* Duch.). *Revista Brasileira de Genética* **8**, 175-182

Mandal A (2006) Studies on genetic diversity, yield components, fruit quality traits and single plant selection approach in pumpkin (*Cucurbita moschata* Duch. Ex. Poir). PhD Thesis, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India, 110 pp

Maynard DN, Carle RB, Elmstrom GW, Ledesma N, Campbell-RJ (2001) Development of hybrid short vine tropical pumpkins. *Proceedings of the InterAmerican Society for Tropical Horticulture* **44**, 1-4

McCreight JD (1984) Tolerance of *Cucurbita* species to squash leaf curl. *Report of the Cucurbit Genetics Cooperative* **7**, 71-72

Mohanty BK (2001) Studies on correlation and path analysis in pumpkin (*Cucurbita moschata*). *Haryana Journal of Horticultural Science* **30**, 86-89

Mohanty BK, Mishra RS (1999) Variation and genetic parameters of yield and its components in pumpkin. *Indian Journal of Horticulture* **56**, 433-434

Mohanty BK, Prusti AM (2002) Heterosis and combining ability for polygenic characters in pumpkin. *Indian Agriculturist* **46**, 27-36

Moura M da CCL, Zerbini FM, da Silva DJH, de Queiróz MA (2005) Identification of pumpkin populations as resistance sources against the zucchini yellow mosaic virus (ZYMV). *Horticultura Brasileira* **23**, 206-210

Munger HM (1990) Availability and use of interspecific populations involving *C. moschata* and *C. pepo*. *Report of the Cucurbit Genetics Cooperative* **13**, 49

Munger HM, Washek RL (1983) Progress and procedures in breeding CMV resistant *C. pepo* L. *Report of the Cucurbit Genetics Cooperative* **6**, 82-83

Muniyappa V, Maruthi MN, Babitha CR, Colvin J, Briddon RW, Rangaswamy KT (2003) Characterization of pumpkin yellow vein mosaic virus from India. *Annals of Applied Biology* **142**, 323-331

Murkovic M, Mulleder U, Neunteufl H (2002) Carotenoid content in different varieties of Pumpkins. *Journal of Food Composition and Analysis* **15**, 633-638

Nee M (1990) The domestication of *Cucurbita* (Cucurbitaceae). *Economic Botany* **44**, 56-68

Pandey S, Singh J, Upadhyay AK, Ram D (2002) Genetic variability for antioxidants and yield components in pumpkin (*Cucurbita moschata* Duch. ex. Poir). *Vegetable Science* **29**, 123-126

Pandit MK, Hazra P, Dutta AK (2006) Study on heterosis for yield and quaility characters of pumpkin (*Cucurbita moschata* Duch. ex. Poir). In: Ram HH, Singh HP (Eds) *Cucurbits Breeding and Production Technology*, Proceedings of the National Seminar on Cucurbits, College of Agriculture, Pantnagar, Uttaranchal, India, pp 319-327

Paran I, Shifriss C, Raccah B (1989) Inheritance of resistance to Zucchini yellow mosaic viorus in the interspecific cross *C. maxima* × *C. ecuadorensis. Euphytica* **42**, 227-232

Paris HS, Cohen S, Burger Y, Yoseph R (1988) Single-gene resistance to Zucchini yellow mosaic virus in *C. moschata. Euphytica* **37**, 27-29

Paris HS, Norson H, Burger Y (1985) Precocious PI 165561 and precocious PI 165561 R pumpkin breeding lines. *HortScience* **20**, 778-779

Paris HS, Brown RN (2005) The genes of pumpkin and squash. *HortScience* **40**, 1620-1630

Pearson OH, Hopp R, Bohn GW (1951) Notes on species crosses in *Cucurbita*. *Proceedings of the American Society for Horticultural Science* **57**, 310-322

Pexioto N, Filgucira FAR, Casali VWD (1990) Development and evaluation of squash (*C. moschata*) lines of the Baianinh group. *Horticultura Brasileira* **8**, 7-10

Pink DAC (1987) Genetic control of resistance to cucumber mosaic virus in *C. pepo*. *Annals of Applied Biology* **111**, 425-432

Provvidenti R (1997) New American summer squash cultivars possessing a high level of resistance to a strain of zucchini yellow mosaic virus from China. *Report of the Cucurbit Genetics Cooperative* **20**, 57-58

Provvidenti R, Robinson RW, Munger HM (1978) Resistance in feral species to six virus infecting *Cucurbita. Plant Disease Reporter* **62**, 326-329

Rahman SM, Hossain M, Islam R, Joarder OI (1993) Plant regeneration from internode segments of *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch. *Current Science* **65**, 562-564

Rai M, Pandey S, Ram D, Kumar S, Singh M (2006) Cucurbits Research in India: An Overview In: Ram HH, Singh HP (Eds) *Cucurbits Breeding and Production Technology*, Proceedings of the National Seminar on Cucurbits, College of Agriculture, Pantnagar, Uttaranchal, India, pp 1-20

Rajan S, Markose B (2001) Pumpkin. In: Thamburaj T, Singh N (Eds) *Text Book of Vegetable, Tuber Crops and Spices*, ICAR, New Delhi, India, pp 292-300

Ramos SRR, Queiróz MA, de Casali VWD, Cruz CD (2000) Genetic diversity of squash germplasm from northwast of Brazil. *Horticultura Brasileira* **18**, 195-199

Rakoczy-Trojanowska M, Malepszy S (1986) Obtaining of hybrids within the family Cucurbitaceae by *in vitro* culture of embryos. I. Characteristics of hybrids from *C. maxima* × *C. pepo*). *Genetica Polonica* **27**, 259-272

Rhodes AM (1959) Species hybridization and interspecific gene transfer in the genus *Cucurbita*. *Proceeding of the American Society for Horticultural Science* **74**, 546-552

Rhodes AM (1964) Inheritance of powdery mildew resistance in the genus *Cucurbita*. *Plant Disease Reporter* **48**, 54-55

Ribeiro A, da Costa CP (1989) Inheritance of mottled leaf in *Cucurbita moschata*. *Report of the Cucurbit Genetics Cooperative* **12**, 70-71

Robinson RW (2000) Rationale and methods for producing hybrid cucurbit seed. In: Basra AS (Ed) *Hybrid Seed Production in Vegetables: Rationale and Methods in Selected Crops*, Food Product Press, India, pp 1-47

Robinson RW, Weeden NF, Provvidenti R (1988) Inheritance of resistance to zucchini yellow mosaic virus in the interspecific cross *C. maxima* × *C. ecuadorensis*. *Report of the Cucurbit Genetics Cooperative* **11**, 74-75

Salama EA, Sill WH Jr. (1968) Resistance to Kansas squash mosaic virus strains among *Cucurbita* species. *Transactions of the Kansas Academy of Science* **71**, 62-68

Sarı N, Yetisir H, Bal U (2006) Parthenogenic haploid induction via irraditated pollen, dihaploidization and ploidy evel determination in vegetable crops. In: Teixeia da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol II), Global Science Books, London, pp 376-384

Sarowar S, Oh HY, Hyung NI, Min BW, Harn CH, Yang SK, Ok SH, Shin JS (2003) *In vitro* micropropagation of a *Cucurbita* interspecific hybrid cultivar – a root stock plant. *Plant Cell, Tissue and Organ Culture* **75**, 179-182

Scott GW (1934) Observations on some inbred lines of bush types of *Cucurbita pepo*. *Proceedings of the American Society for Horticultural Science* **32**, 480

Seo YK, Paek KY, Hwang JK, Cho YH (1991) Micropropagation of root stock and production of interspecific hybrids by embryo culture in *Cucurbita*. *Jour-nal of the Korean Society for Horticultural Science* **32**, 137-145

Sharma JR, Kumar JC (1995) Pumpkin varieties suitable for ketchup. *Punjab Vegetable Grower* **30**, 64-65

Shifriss O (1985) Origin of gynoecism in squash. *HortScience* **20**, 889-891

Shifriss O (1981) Origin, expression and significance of gene B in *Cucurbita pepo*. *Journal of the American Society for Horticultural Science* **106**, 220-232

Shifriss O (1991a) Expression of B genes in *Cucurbita*. *Report of the Cucurbit Genetics Cooperative* **14**, 113-115

Shifriss O (1991b) The two B genes of Cucurbita are unlinked. *Report of the Cucurbit Genetics Cooperative* **14**, 116-122

Shifriss O (1993) A case of extremely low expressivity of gene B2 in *C. moschata*. *Report of the Cucurbit Genetics Cooperative* **16**, 64-67

Shifriss O, Volin RB, Williams TV (1989) Relationship between B genes of two *Cucurbita* species. *Report of the Cucurbit Genetics Cooperative* **12**, 75-78

Singh HP (2006) An overview of cucurbits research. In: Ram HH, Singh HP (Eds) *Cucurbit Breeding and Production Technology*, Proceedings of the National Seminar on Cucurbits, College of Agriculture, Pantnagar, Uttaranchal, India, pp 21-40

Singh SP, Rhodes AM (1961) A morphological and cytological; study of male sterility in *C. maxima*. *Proceeding of the American Society for Horticultural Science* **78**, 375

Sirohi PS (1993) Genetic diversity in cucurbits, pumpkin (*Cucurbita moschata* Duch. ex. Poir.). *Indian Horticulture* **38**, 35-37

Sirohi PS, Choudhury B, Kalda TS (1991) Pumpkin 'Pusa Vishwas' for tropical and subtropical growing. *Indian Horticulture* **36**, 24-26

Sirohi PS, Kumar TS, Choudhury B (1985) Studies on heterosis in pumpkin (*C. moschata*). *South Indian Horticulture* **33**, 297-301

Sirohi PS, Kumar TS, Choudhury B (1986) Diallel analysis for variability in *Cucurbita moschata* (Duch) Poir. *Indian Journal of Agricultural Science* **56**, 171-173

Sirohi PS, Reddy YS, Behera TK (2002) Heterosis for yield and its attributing traits in pumpkin (*Cucurbita moschata* Duch. ex. Poir.). *Vegetable Science* **29**, 178-179

Sirohi PS, Yayasani SR (2001) Gene action of mineral elements and vitamins in pumpkin (*Cucurbita moschata*). *Vegetable Science* **28**, 127-129

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 Science* **165**, 663-669

Sitterly WR (1972) Breeding for disease resistance in cucurbits. *Annual Review of Phytopathology* **10**, 471-490

Sohrab SS, Mandal B, Ali A, Varma A (2006) Molecular diagnosis of emerging begomovirus diseases in cucurbits occurring in northern India. *India Journal of Virology* **17**, 88-95

Sowell G, Corley WL (1973) Resistance to *Cucurbita* plant introductions to powdery mildew. *HortScience* **8**, 492-493

Srivastava U (2006) Genetic resource management in cucurbits. In: Ram HH, Singh HP (Eds) *Cucurbits Breeding and Production Technology*, Proceedings of the National Seminar on Cucurbits, College of Agriculture, Pantnagar, Uttaranchal, India, pp 41-64

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK (2005) Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non B biotype that can induce silverleaf symptoms in squash in Uganda. *Annals of Applied Biology* **147**, 253-265

Swarup V (2006) *Vegetable Science and Technology in India*, Kalyani Publishers, New Delhi, India, pp 388-396

Vucetic J, Cirovic M, Matic V (1989) Chemical composition, nutritive value and healing properties of the pumpkin (*Cucurbita maxima* Duch.). *Hrana I Ishrana* **30**, 159-161

Wang JJ, Yeh SD (1998) Characterization of the papaya ringspot virus W type isolates collected from different areas of Taiwan by host reactions, immunodiffusion tests and RT-PCR. *Plant Protection Bulletin Taipei* **40**, 383-395

Watterson JC, Williams PH, Durbin RD (1971) Response of *Cucurbita* to *Erwinia trachiiphilla*. *Plant Disease Reporter* **55**, 816-819

Weeden NF (1984) Isozyme studies indicate that the genus *Cucurbita* is an ancient tetraploid. *Report of the Cucurbit Genetics Cooperative* **7**, 84-85

Wessel-Beaver L (1993) Powdery and downy mildew resistance in *Cucurbita moschata* accessios. *Report of the Cucurbit Genetics Cooperative* **16**, 73-74

Wessel-Beaver L, Katzir N (2000) Inheritance of silverleaf resistance in *Cucurbita moschata*. *Acta Horticulturae* **510**, 289-295

Whitaker TW (1959) An interspecific cross in *Cucurbita* (*C. lundelliana* × *C. moschata*). *Madrono* **15**, 4-13

Whitaker TW, Bemis WP (1976) Cucurbits. In: Simonds NN (Ed) *Evolution of Crop Plants*, Longman, London, pp 64-69

Whitaker TW, Cutler HC (1971) Pre-historic cucurbits from the valley of Oaxaca. *Economic Botany* **25**, 123-127

Whitaker TW, Davis GN (1962) *Cucurbits, Botany, Cultivation and Utilization*, World Crops Books, Interscience Publishers, New York, 250 pp

Whitaker TW, Knight RJ Jr. (1980) Collecting cultivated and wild cucurbits in Mexico. *Economic Botany* **34**, 312-319

Whitaker TW, Robinson RW (1986) Squash Breeding. In: Bassett MJ (Ed) *Breeding Vegetable Crops*, AVI Publishing Company, Westport, Connecticut, USA, pp 209-242

Wilson HD (1989) Discordant patterns of allozyme and morphological variation in Mexican *Cucurbita*. *Systematic Botany* **14**, 612-623

Wilson HD (1990) Gene flow in squash species. *BioScience* **40**, 449-455

Wilson HD, Doebley J, Duvall M (1992) Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae). *Theoretical and Applied Genetics* **84**, 859-865

Wu ZR, Jin TM (1998) Determination of β-carotene in different pumpkin varieties by HPLC. *Acta Agriculturae Boreali Sinica* **13**, 141-144

Yamaguchi J, Shiga T (1993) Characteristics of regenerated plants via protoplast electrofusion between melon (*Cucumis melo*) and pumpkin (interspecific hybrid, *Cucurbita maxima* × *C. moschata*). *Japanese Journal of Breeding* **43**, 173-182

You BJ, Chiang CH, Chen LF, Su WC, Yeh SD (2005) Engineered mild strains of papaya ringspot virus for broader cross protection in cucurbits. *Phytopathology* **95**, 533-540

Youn SJ, Chung HD (1998) Genetic relationship among the local varieties of the Korean native squashes (*Cucurbita moschata*) using RAPD technique. *Journal of the Korean Society for Horticultural Science* **39**, 517-521

Zhang XG, Liu PY (1998) Protoplast fusion between *Cucumis sativa* and *Cucurbita moschata* or *C. ficifolia*. *Journal of Southwest Agricultural University* **20**, 293-297

Zhang YJ, Li MF, Xiang N, Li GF (2006) Identification and the analysis on characterization of the 3′ terminal sequences of the genome of zucchini yellow mosaic virus. *Acta Phytophylacica Sinica* **33**, 32-36

Zhou XL (1987) A study on the breeding of naked Kernel pumpkin and its genetic behaviour. *Acta Horticulturae Sinica* **14**, 115-118