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ORIGINAL ARTICLES

Leaf Reduced Sugar Content and RAPD Analyses Potentially Typify Pumpkins (*Cucurbita spp.*) Flowering Response and Productivity

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ABSTRACT

In a preliminary assessment, eight cultivar germplasm including seven Egyptian landraces of *Cucurbita moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') and of *C. maxima* ('Kafr El-Battikh-2' 'Faraskour' and El-Zarka") and the exotic cv 'Connecticut Field' (*C. pepo*) were planted on May in the summer season. The Egyptian landraces of *C. maxima* and the exotic cv of *C. pepo* produced no fruits at all. Reduced sugar contents determined in leaves of their 15- and 30-day-old plants were only one third of those found in leaves of Egyptian landraces of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus'). Subsequently, seven exotic cvs belong to *C. maxima* ('Atlantic Giant', 'Big Max' and 'Amish pie', *C. pepo* ('Cheyenne' and 'Cornfield'), *C. moschata* ('Early Butternut') and *C. mixta* ('Cushaw') in addition to the abovementioned cultivar germplasm were tested during both summer (March and April) and autumn (Aug. and Sept.) plantings. Consistently, pumpkins having higher reduced sugar contents in their leaves especially at flower anthesis and beginning of fruit-set (30 days after planting) characterized by producing higher fruit yield. This is considered new information that would enable further improvement of pumpkin crop production. Pumpkins planted on August, commonly, produced the highest fruit yield followed by planting on March. Overall results for fruit yield, fruit flesh contents of TSS and dry matter and fruit storability showed that the Egyptian landraces 'Kafr El-Battikh' and 'Kafr Saad' and the exotic cv 'Early Butternut' (all belong to *C. moschata*) were the most elite cultivars to grow for production in southern Egypt and other regions of similar climatic condition. RAPD analysis was conducted using three diverse cvs in flowering response ['Kafr El-Battikh-1' (normally flowered and set fruits, *C. moschata*), 'El-Zarka' (flower buds formed but did not open, *C. maxima*) and cv 'Connecticut Field' (flowers opened but did not set fruits, *C. pepo*)]. Cluster dendrogram showed that the cultivar 'Kafr El-Battikh-1' was separated in a single branch from the other two cultivars reflecting a relatively greater genetic distance from them. In agreement with the flowering responses of the three studied germplasm, fragment of molecular size 693 bp generated by OPA06 primer and fragment of molecular size 848 bp generated by OPA04 primer appeared only in 'Kafr El-Battikh' and 'Connecticut field'. These fragments are considered as positive specific markers for flowering response. These results obtained from RAPD analysis, in general, suggested that molecular marker approach could be considerably potential for discriminating pumpkin cultivars concern both their genetic relationship and their functional flowering response and fruiting productivity.

***Key words*:** landraces, *maxima*, *mixta*, *moschata*, *pepo*, crop production.

Introduction

Pumpkins are cucurbitaceous crop of genus *Cucurbita* that are grown for production of mature fruits. They may belong to one of four species (*moschata*, *maxima, mixta* and *pepo*). This crop requires relatively long (3 to 6 months) warm (18 to 30 °C) growing season for its optimum production (Hassan, 2004). It has been well documented that fruit yield of pumpkins is substantially affected by prevailing climatic conditions (Knott, 1958; Devlin and Witham, 1983; Wien, 1997; Hassan, 2004) including temperature, day length and light intensity. Differential response to such climatic conditions is exhibited by diverse species and cultivars (Wien, *et al.,* 2002). Some cultivars may qualitatively affected showing no flower bud formation or no flower anthesis or no fruit set (Wien, 1997; Wien *et al.,* 2002 and Mostafa, 2006). Others may quantitatively respond showing varying degrees of depression in fruit yield (Mostafa, 2006 and Hussein *et al.,* 2010). Therefore, literature shows renewable research interest in studying cultivar response to different planting season and dates in various regions around the World (Damarany *et al.,* 1995 and Mostafa, 2006 in Egypt and Wein *et al.,* 2002 in USA). In this context, landrace cultivars adapted to certain conditions prevailing in a given region may be of special usefulness for pumpkins production (Wien, 1997).

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Flower bud initiation and sex determination occur at early stage of cucurbitaceous plant growth (Hassan, 2008) and could be modified by application of plant growth regulators (Wien, 1997; Wien *et al.,* 2004; Chatterjee and Satya, 2006). Presumably translocation/partitioning and accumulation of primary photosynthetic assimilate and its availability in plant leaves would be in action. However, we found no clear information in literature on this notion as coupled with pumpkins fruit yield. Unlike cucurbitaceous crop that grown for production of immature fruits (Mohamed, 1996), fruit yield of pumpkins is largely affected by fruit size as they harvested mature. Fruit crop, therefore, may be controlled by availability of assimilates for translocation into fruit sink. The objective of the present study was to assess reduced sugar contents in leaves along with fruit yield and quality for 15 collections of pumpkin cultivar germplasm when grown on diverse planting dates in summer and autumn seasons. Random amplified polymorphic DNA (RAPD) analysis was used to typify the genetic differences and relationships among chosen pumpkin cultivars representing dissimilarity found in the studied pumpkin germplsam regarding the expression of functional flowering and enhanced fruit yielding.

Materials and Methods

A three-year field trial (2008, 2009 and 2010) were executed in clay soil, at the Experimental Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt, to evaluate flowering response and fruit yield of some pumpkin germplasm when grown in different planting dates and seasons. In a preliminary assessment, eight cultivar germplasm including seven Egyptian landraces of *Cucurbita moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') and of *C. maxima* ('Kafr El-Battikh-2' 'Faraskour' and El-Zarka") and the exotic cv 'Connecticut Field' (*C. pepo*) were planted on May in the summer season. Subsequently, seven exotic cvs belong to *C. maxima* ('Atlantic Giant', 'Big Max' and 'Amish pie', *C. pepo* ('Cheyenne' and 'Cornfield'), *C. moschata* ('Early Butternut') and *C. mixta* ('Cushaw') in addition to the abovementioned cultivar germplasm were tested during both summer (March and April) and autumn (Aug. and Sept.) plantings in 2009 and 2010. Land preparation and all cultural practice were done as recommended for production of pumpkins (Hassan, 2004). The tested pumpkin cultivars are presented in Table (1) with their common names, source and the species to which they belong. Seeds during the whole course of the study were from the same seed lots. The experiments were laid out as simple experiment in randomized complete-blocks (RCBD) with four replicates.

Table 1: Name, species and source of fifteen pumpkin cultivar germplasm evaluated in this study.

Serial No.	Name	Genus	Species	Source
1	'Kafr El-Battikh-1'	*Cucurbita*	*moschata*	Local growers, Egypt (Demietta)
2	'Kafr Saad'	*Cucurbita*	*moschata*	Local growers, Egypt, (Demietta)
3	'El-Edua'	*Cucurbita*	*moschata*	Local growers, Egypt, (El-Minia)
4	'Qus'	*Cucurbita*	*moschata*	Local growers, Egypt, (Qena)
5	'Early Butternut'	*Cucurbita*	*moschata*	Heirloom seeds Co. W. USA
6	'Kafr El-Battikh-2'	*Cucurbita*	*maxima*	Local growers, Egypt, (Demietta)
7	'Faraskour'	*Cucurbita*	*maxima*	Local growers, Egypt, (Demietta)
8	'El-Zarka'	*Cucurbita*	*maxima*	Local growers, Egypt (Demietta)
9	'Atlantic Giant'	*Cucurbita*	*maxima*	Heirloom seeds Co. W. USA
10	'Amish pie'	*Cucurbita*	*maxima*	Heirloom seeds Co. W. USA
11	'Big Max'	*Cucurbita*	*maxima*	Heirloom seeds Co. W. USA
12	'Connecticut Field'	*Cucurbita*	*pepo*	Heirloom seeds Co. W. USA
13	'Cheyenne'	*Cucurbita*	*pepo*	Heirloom seeds Co. W. USA
14	'Cornfield'	*Cucurbita*	*pepo*	Heirloom seeds Co. W. USA
15	'Cushaw'	*Cucurbita*	*mixta*	Heirloom seeds Co. W. USA

Leaf Reduced Sugar Content and Fruit Yield Analyses:

Data were recorded for all cultivars in this study with regard to reduced sugar content (mg/100g fresh leaf tissue) of 15- and 30-day old plants (Dobois, *et al.,* 1956), number of opened female flowers per plant, number of opened male flowers per plant, fruit set percentage, main vine length (cm) of mature plants, yield per plant (kg), average fruit weight (kg), number of fruits per plant and fruit shelf life (days) at normal room temperature. Fruit dry matter was determined as fresh weight minus dry weight (70 ˚C in a fan electric oven until constant weight) multiplied by 100. Total soluble solids (TSS) of fruit flesh juice were determined using Carl Zeiss hand refractometer, (A.O.A.C., 1965). All data of this study were subjected to combined analysis of variance over planting dates and years according to Gomez and Gomez (1984). Means of pumpkin cultivar germplasm were compared using Dancan Multiple Range Test (DMRT). Means of planting dates were compared using the Least Significant Difference (LSD) Test at 0.05 probability level.

Random Amplified Polymorphic DNA (RAPD) Analysis:

RAPD analysis was conducted using three pumpkin cultivars. These cvs were selected to represent the dissimilarity found in the studied pumpkin germplsam regarding the expression of functional flowering and enhance fruit yielding.

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DNA Extraction:

Total genomic DNA was isolated from fresh leaves, bulked from 5 different plants per genotype using CTAB protocol (Murray and Thompson, 1980) with some modifications. RNA was removed during the DNA preparation by adding 10 µl of RNAase (10mg/ml) and then incubated for 30 min at 37°C. DNA concentration was quantified using a spectrophotometer (Genova-2138).

RAPD-PCR Reactions:

Six RAPD primers (Table 2), obtained from (metabion international AG), were tested to amplify the templated DNA. The reaction conditions were optimized and mixtures (25 µl total volume) composed of 11.0 µl dH_2O, 3.0 µl 10X reaction buffer, 3.0 µl dNTP's mix, 2.0 µl primer, 4.0 µl $MgCl_2$, 0.3 µl Taq DNA polymerase and 1 µl Template DNA. Amplification condition were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K.) with the following specification: initial denaturation for 3 min at 94ºC (1^{st} step), 40 cycles of 1 min at 92ºC, 1 min at 33ºC and 2 min at 72ºC (2^{nd} step), 10 min at 72ºC (3^{rd} step), then followed by a final hold at 4ºC.

Table 2: Primer sequences and codes used.

Serial No.	Primer codes	Sequence (5’ to 3’)
1	OPA04	5'-TGCCGAGCTG-3'
2	OPA06	5'-GGTCCCTGAC-3'
3	OPA07	5'-GTGACGTAGG-3'
4	OPB05	5'-TGCGCCCTTC-3'
5	OPA05	5'-AGGGGTCTTG-3'
6	OPZ15	5'-CAGGGCTTTC -3'

RAPD Data Analysis:

RAPD-based molecular markers were scored visually using the software package MVSP (Multi-Variate Statistical Package) and DNA bands were scored as present (1) or absent (0). The pairwise comparisons between the tested isolates were used to calculate the coefficient of genetic similarity matrix (Gs) according to Nei and Li (1979). To convert the genetic similarity into genetic distance, logarithmic transformation (-Ln Gs) was computed to linearize the distance measure. Cluster analysis was presented as the dendrogram based on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA).

Results and Discussion

Leaf Reduced Sugar Content And Fruit Yield Analyses:

Determination of reduced sugar contents in the leaves of 15- and 30-day-old pumpkin plants (Tables 3 A and B) in the present study provided meaningful insight on pumpkins growth (Table 4A), flowering response (Tables 4B and 5A), fruit set (Table 6 A) and fruit yield (Table 5B) and its main components (Tables 6B, 7A and B and 8A). The Egyptian landraces of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') produced their highest fruit yield when grown on August. Reduced sugar content in the leaves determined 15 days after planting was not the highest among those of the other plantings (March, April, May and Sept.). This would rationally be ascribed to relative high temperature prevailing during Aug (Fig. 1). With the advent of Sept., available sugar contents elevated as shown by 30-day-old flowering plants. This result suggest that availability of simple photosynthetic assimilates is crucial during flower anthesis and fruit setting. Comparing to March planting, increased fruit yield for Aug. planting was exhibited in terms of increased number and weight of harvested mature fruits.

Planting the Egyptian landraces of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') on March was the second after Aug. to produced high pumpkins fruit yield (Table 5B). Reduced sugar contents in leaves of 15-day-old plants grown during March were higher than those of Aug. planting date (Table 3A). On contrast, leaves of 30-day-old plants of March planting (Table 3B) had lower content of reduced sugar as compared to those planted on Aug. This could be attributed to elevated temperature with the introduction of April. Flowering plants of March planting date formed reduced number of pistillate but increased number of staminate flowers. The notion on the prominence of photosynthetic assimilates availability status during flower anthesis and fruit setting is substantiated by both Aug., and March planting data of the Egyptian landraces of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus'). Discriminately from other planting dates of

lower cropping (April, May and Sept.), fruit yield for March planting was rather resulted from increased fruit weight in compensation of the number of harvested mature fruits (Table 6B).

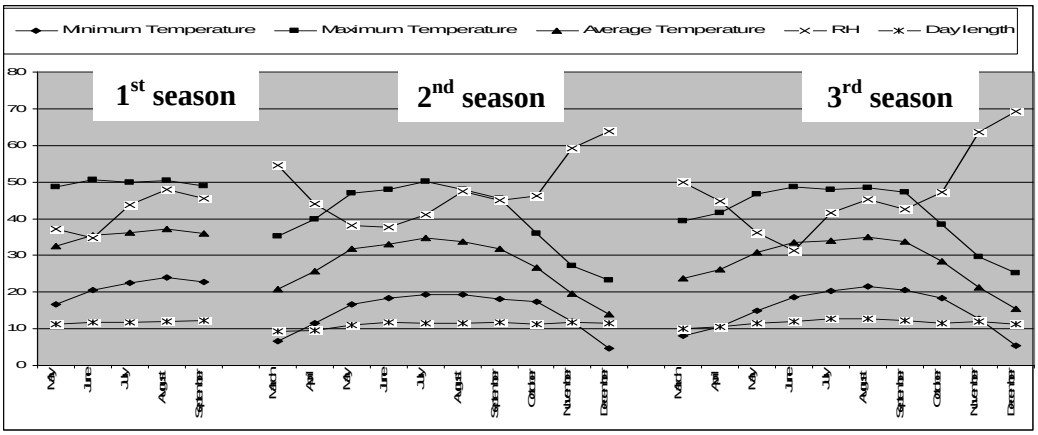

Fig. 1: Minimum, Maximum and average day temperature (c), Relative Humidity (RH%) and day length (h) during experiment conduct period of time (Assiut Univ. Agric. Meteorological Station).

Table 3: Average content of reduced sugar (mg/100g) of fresh leaves (15 and 30 days from sowing) produced from pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons[1].

Planting dates	May-01	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
Seasons	1st season	2nd season				3rd season			
Cultivars									
A- Average content of reduced sugar (mg/100g) of fresh leaves (15 days from sowing)									
'Kafr El-Battikh-1'	12.6 b[2]	24.5 a	16.0 a	22.7 a	26.9 a	23.2 a	15.1 a	21.3 a	25.4 a
'Kafr Saad'	12.4 b	23.8 ab	15.6 a	20.4 b	26.1 ab	22.4 a	14.8 a	18.7 b	24.6 ab
'El-Edua'	12.0 c	23.2 b	15.5 a	20.8 b	25.4 b	21.9 a	14.7 a	18.6 b	24.0 b
'Qus'	12.9 a	23.3 b	16.3 a	20.8 b	25.5 b	22.0 a	15.4 a	19.7 b	24.1 ab
'Early Butternut'	-[3]	19.0 c	11.6 b	16.8 c	18.4 c	18.0 b	11.9 b	13.2 c	17.4 c
'Kafr El-Battikh-2'	3.0 e	4.7 ef	3.2 d	3.5 fg	4.5 efg	4.4 de	3.0 cd	3.3 def	4.3 ef
'Faraskour'	2.5 f	3.9 f	2.8 d	2.8 g	3.8 fg	3.7 e	2.7 cd	2.9 f	3.6 f
'El-Zarka'	2.6 f	3.8 f	3.0 d	3.3 fg	3.7 fg	3.6 e	2.8 cd	3.1 ef	3.5 f
'Atlantic Giant'	-	3.8 f	2.7 d	2.7 g	3.7 fg	3.6 e	2.6 d	2.6 f	3.5 f
'Amish pie'	-	3.7 f	3.2 d	3.5 fg	3.6 g	3.5 e	3.0 cd	3.4 def	3.4 f
'Big Max'	-	3.6 f	3.0 d	3.3 fg	3.5 g	3.4 e	2.8 cd	3.1 ef	3.3 f
'Connecticut Field'	4.1 d	6.4 d	4.3 c	5.2 d	6.0 d	6.0 c	4.0 c	4.5 de	5.6 d
'Cheyenne'	-	5.7 de	4.4 c	4.9 de	5.3 de	5.4 cd	4.1 c	4.6 d	5.0 de
'Cornfield'	-	5.1 e	3.2 d	3.5 fg	4.8 ef	4.8 cde	3.0 cd	3.3 def	4.5 def
'Cushaw'	-	4.8 ef	3.7 cd	4.1 ef	4.4 fg	4.5 de	3.5 cd	3.9 def	4.2 ef
$LSD_{0.05}$:[4]		0.5				0.7			
B- Average content of reduced sugar (mg/100g) of fresh leaves (30 days from sowing)									
'Kafr El-Battikh-1'	17.4 a	35.8 a	23.4 a	39.2 a	33.1 a	33.9 a	22.0 a	37.0 a	31.1 a
'Kafr Saad'	14.3 b	34.7 ab	22.8 a	38.0 ab	29.8 b	32.8 a	21.5 a	36.0 ab	27.4 b
'El-Edua'	14.7 b	33.9 b	22.6 a	37.1 b	30.3 b	32.0 a	21.4 a	35.1 b	27.2 b
'Qus'	14.6 b	34.0 b	23.8 a	37.2 b	30.4 b	32.1 a	22.5 a	35.2 ab	28.7 b
'Early Butternut'	-	27.8 c	17.0 b	26.9 c	24.5 c	26.2 b	17.4 b	25.4 c	19.3 c
'Kafr El-Battikh-2'	4.1 d	7.2 efg	4.9 de	7.0 ef	5.4 fg	6.8 de	4.6 cd	6.6 def	5.1 def
'Faraskour'	3.9 e	6.0 fg	4.4 e	5.8 f	4.4 g	5.7 e	4.2 cd	5.5 ef	4.5 ef
'El-Zarka'	4.3 d	5.9 g	4.6 e	5.7 f	5.0 fg	5.6 e	4.3 cd	5.4 ef	4.8 def
'Atlantic Giant'	-	5.9 g	4.2 e	5.7 f	4.2 g	5.6 e	4.0 d	5.4 ef	3.9 f
'Amish pie'	-	5.7 g	4.9 de	5.5 f	5.5 fg	5.4 e	4.7 cd	5.2 f	5.2 def
'Big Max'	-	5.6 g	4.6 e	5.5 f	5.1 fg	5.3 e	4.4 cd	5.2 f	4.8 def
'Connecticut Field'	5.6 c	9.4 d	6.3 cd	8.8 d	7.8 d	8.9 c	6.0 cd	8.4 d	6.6 de
'Cheyenne'	-	8.4 de	6.5 c	7.8 de	7.2 de	8.0 cd	6.1 d	7.4 de	6.8 d
'Cornfield'	-	7.5 ef	4.7 e	7.0 ef	5.2 fg	7.1 cde	4.4 cd	6.6 def	4.9 def
'Cushaw'	-	7.0 efg	5.5 cde	6.6 ef	6.1 ef	6.7 de	5.2 cd	6.2 ef	5.8 def
$LSD_{0.05}$:		0.7				1.0			

[1] year, season and planting date interaction was significant.
[2] means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).
[3] not grown.
[4] to compare means of same cultivar grown on different planting dates.

The Egyptian landrace pumpkins of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') planted on April and May showed progressive decline in reduced sugar contents in the leaves 15 (Table 3A) and 30 (Table 3B) days after planting as affected by raised temperature with the advent of May and June, respectively. Those pumpkins grown during April through May exhibited decreased plant length (Table 4A), number of female flowers (Table 4B) and fruit weight (Table 7A) while forming increased number of male flowers (Table 5A). Eventually they had significantly lower fruit yield (Table 5B) than Aug., and March plantings. When grown during May through June, the Egyptian landrace pumpkins of *C. moschata* ('Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus') produced the least fruit yield among all other planting dates. The situation was more serious showing no fruit crop at all in *C. maxima* (Egyptian landraces, namely, 'Kafr El-Battikh-2', 'Faraskour' and 'El-Zarka') and *C. pepo* pumpkins (exotic, cv 'Connecticut Field') where either no flower buds were opened or flowers opened but did not set fruits. Noticeably, the available reduced sugar contents in leaf tissues of 30-day-old flowering plants for this germplasm ('Kafr El-Battikh-2', 'Faraskour' and 'El-Zarka') and 'Connecticut Field') were only about one-third those of 'Kafr El-Battikh-1', 'Kafr Saad', 'El-Edua' and 'Qus'. It seemed, therefore, that availability of certain level of reduced sugar contents is crucial during.

Flower anthesis and fruit development. Also, it is suggested that crop production under adverse climatic (high temperature and light intensity) in late summer planting is a pumpkin species-dependant (Wien *et al.,* 2002). Cultivar germplasm of *C. maxima* and *C. pepo* showed a qualitative pattern of response to climatic conditions in late summer planting (May) while quantitative pattern was found in *C. moschata*. This latter species seemed to be more adapted to such extreme climatic condition (Hussein, *et al.,* 2010). Differential fruit yield shown by *C. moschata* pumpkins in all planting dates indicates that its production is also cultivar-dependant. Egyptian landraces 'Kafr El-Battikh-1' and 'Qus' were the greatest cropping pumpkins of specimen *moschata* and also overall other specimens

Table 4: Average plant length (cm) at 90 days after planting date and number of opened female flowers per plant produced from pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons[(1)].

Planting dates Seasons Cultivars	May-01 1st season	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
		2nd season				3rd season			
A- Average plant length (cm) at 90 days after planting date									
'Kafr El-Battikh-1'	280.6a[(2)]	438.9a	329.8a	480.9a	366.0a	415.0a	310.5a	454.1a	345.1a
'Kafr Saad'	273.7a	425.6a	320.8ab	466.3a	356.0a	401.8a	303.6ab	441.2a	335.6a
'El-Edua'	267.0a	415.3a	319.0ab	454.9a	354.0a	392.1a	301.7ab	430.1a	333.7a
'Qus'	287.4a	416.6a	335.8a	456.3a	372.6a	393.4a	317.0a	431.5a	352.2a
'Early Butternut'	-[(3)]	301.3c	226.3f	329.6d	251.1d	284.7c	213.3f	311.6d	236.2d
'Kafr El-Battikh-2'	267.6a	414.4a	316.5ab	454.0a	351.2a	391.9a	299.2ab	428.9a	331.8a
'Faraskour'	194.2b	345.8b	232.5ef	378.4b	258.0d	326.6b	219.1ef	357.7h	244.3d
'El-Zarka'	187.3b	340.3bc	271.3cde	372.4bc	301.1bc	321.1bc	256.3cde	352.3bc	285.2bc
'Atlantic Giant'	-	338.6bc	244.5def	370.6bcd	271.4cd	319.7bc	231.2def	350.3bcd	255.9cd
'Amish pie'	-	327.5bc	319.8ab	358.4bcd	354.9a	309.6bc	302.1ab	338.5bcd	335.6a
'Big Max'	-	322.8bc	299.4abc	353.2bcd	332.3ab	304.9bc	282.7abc	333.9bcd	313.9ab
'Connecticut Field'	272.4a	408.4a	320.5ab	447.3a	355.7a	385.7a	302.5ab	423.3a	335.6a
'Cheyenne'	-	97.3d	87.5g	105.1e	97.1e	91.9d	82.7g	99.3e	91.8e
'Cornfield'	-	325.9bc	236.5ef	356.6bcd	262.5d	308.2bc	223.6ef	336.8bcd	247.8d
'Cushaw'	-	305.0bc	280.3bcd	333.7cd	311.0b	288.1bc	264.6bcd	315.4cd	294.6b
$LSD_{0.05}$:[(4)]		20.9				20.7			
B- Number of opened female flowers per plant									
'Kafr El-Battikh-1'	9.3 a	11.5 bc	10.0 bc	13.0 ab	16.5 c	10.3 b	8.3 bc	11.5 c	14.8 c
'Kafr Saad'	8.3 b	10.0 d	9.0 c	11.0 c	14.0 d	8.8 c	7.3 c	9.5 d	12.8 d
'El-Edua'	8.3 b	10.3 cd	9.3 bc	11.3 c	14.3 d	9.0 c	7.5 c	10.0 d	13.0 d
'Qus'	9.5 a	12.3 ab	10.5 ab	14.3 a	18.3 b	10.8 b	8.8 b	12.8 b	16.3 b
'Early Butternut'	-	13.5 a	11.8 a	14.0 a	19.8 a	12.3 a	10.3 a	14.0 a	17.8 a
'Kafr El-Battikh-2'	0.0 d	7.5 e	6.5 de	8.5 d	10.5 ef	6.3 de	5.0 de	7.5 e	9.5 ef
'Faraskour'	0.0 d	6.8 ef	5.8 def	7.8 de	9.8 fg	5.5 ef	5.0 de	6.8 ef	8.8 g
'El-Zarka'	0.0 d	5.8 fg	5.0 fg	6.8 ef	8.5 gh	4.8 fg	4.5 def	5.8 fg	7.8 h
'Atlantic Giant'	-	3.8 h	3.5 h	4.8 g	6.0 i	3.8 g	3.3 f	4.3 h	5.8 h
'Amish pie'	-	4.5 gh	4.0 gh	5.5 fg	7.0 i	4.3 fg	3.5 f	4.8 gh	6.5 h
'Big Max'	-	4.5 gh	4.0 gh	5.5 fg	7.0 i	4.3 fg	3.5 f	4.8 fg	6.5 h
'Connecticut Field'	6.0 c	8.0 e	7.0 d	9.0 d	11.5 e	6.8 d	5.5 d	8.0 e	10.5 e
'Cheyenne'	-	5.5 fg	5.3 efg	4.3 g	7.3 hi	5.0 fg	4.5 def	6.0 fg	8.3 fg
'Cornfield'	-	5.8 fg	5.0 fg	6.3 f	9.0 g	5.0 fg	4.0 ef	6.0 fg	8.3 fg
'Cushaw'	-	10.5 cd	9.3 bc	11.8 bc	15.0 d	9.0 c	7.5 c	10.5 cd	13.3 d
$LSD_{0.05}$:		0.2				0.4			

(1) year, season and planting date interaction was significant.

(2) means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).

(3) not grown. (4) to compare means of same cultivar grown on different planting dates.

Leaves of 15-day-old the Egyptian landrace of *moschata* pumpkin plants grown during Sept. had the highest content of reduced sugar among all other plantings. However, lower sugar contents than Aug. planting date was found when determined for 30-day-old plants. This could be a result of generating strong assimilate sink by the increased number of opened female flowers that set fruits. Because of the decreased temperature later during the fruit development, their fruit weight was sharply decreased. Therefore, fruit yield produced from the Egyptian landrace of *moschata* pumpkins planted on Sept. was the lowest among the other planting dates. Worthwhile, the *moschata* pumpkin 'Early Butternut' showed the highest leaf sugar content for 30-day-old plants and produced its highest fruit yield when planted on March. As depicted above the Egyptian landrace of *moschata* produced the highest yield when grown on Aug., followed by March. Plants of cultivar 'Early Butternut' have relatively compact growth and smaller early matured fruits. For these characteristics of 'Early Butternut', its production suited to warm climatic conditions of March to April and approached full fruit development before coming of adverse high temperature of June.

Table 5: Number of opened male flowers per plant and average fruit yield (kg/plant) produced from pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons[1].

Planting dates Seasons Cultivars	May-01 1st season	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
		2nd season				3rd season			
A- Number of opened male flowers per plant									
'Kafr El-Battikh-1'	195.5 [2]	120.3b	162.8a	149.5b	123.3bc	110.0b	138.0a	195.3a	111.8bc
'Kafr Saad'	189.0c	110.8b	157.3a	110.8cd	91.5ef	99.3bc	131.3a	112.0b	85.0cde
'El-Edua'	188.5c	111.5b	159.0a	124.3c	106.0cde	100.5bc	133.5a	114.0b	99.3bcd
'Qus'	165.3d	107.5b	137.0b	151.8b	133.5b	97.5bc	117.8a	178.8a	122.5b
'Early Butternut'	-[3]	22.0g	32.0d	35.3f	30.3g	20.5f	28.8d	36.0e	27.8f
'Kafr El-Battikh-2'	0.0e	85.0c	82.3c	101.0de	116.3bcd	73.5cd	65.0bc	91.8bc	108.8bc
'Faraskour'	0.0e	56.8de	72.3c	78.5e	73.0f	48.8def	65.3bc	80.3cd	70.3de
'El-Zarka'	0.0e	60.5de	88.3c	87.0e	87.3ef	59.3de	81.8b	75.8cd	80.8cde
'Atlantic Giant'	-	56.0de	72.0c	80.8e	86.3ef	58.5de	68.8bc	78.8cd	83.0cde
'Amish pie'	-	59.5de	73.0c	79.3e	88.3ef	58.0de	66.0bc	78.3cd	81.5cde
'Big Max'	-	66.5cde	72.5c	91.3de	100.8de	65.8de	65.0bc	79.0cd	92.5cde
'Connecticut Field'	210.0a	146.8a	177.3a	176.8a	191.0a	162.0a	142.8a	174.5a	197.0a
'Cheyenne'	-	45.8ef	49.0d	38.0f	48.8g	42.5ef	42.8cd	53.8de	61.8e
'Cornfield'	-	72.8cd	84.8c	100.0de	106.3cde	65.3de	70.0bc	85.3bc	91.3cde
'Cushaw'	-	27.0fg	40.3d	42.0f	34.0g	23.5f	33.5d	38.3e	30.5f
$LSD_{0.05}$:[4]		5.8				10.4			
B- Average fruit yield (kg/plant)									
'Kafr El-Battikh-1'	24.918b	44.503a	36.913ab	53.982a	41.117a	35.657a	31.914a	36.023a	30.413a
'Kafr Saad'	20.058c	37.686b	34.973ab	50.179a	31.954b	27.951b	24.917b	32.562a	25.180b
'El-Edua'	20.434c	35.412b	31.662b	34.805b	29.128b	33.494a	23.544b	32.595a	24.585b
'Qus'	27.743a	47.870a	39.575a	50.476a	38.375a	33.002a	26.789b	34.742a	27.987ab
'Early Butternut'	-	2.526e	1.769c	2.006f	2.087e	3.408g	2.087e	2.349e	1.426f
'Kafr El-Battikh-2'	0.000d	15.444c	8.624c	16.780cd	11.045cd	19.989c	16.286c	21.352b	10.494cd
'Faraskour'	0.000d	8.917de	8.099c	19.741c	17.169c	14.836d	8.813d	16.379c	12.108c
'El-Zarka'	0.000d	6.716de	5.419c	10.829de	8.182de	10.981de	9.004d	10.553d	7.178de
'Atlantic Giant'	-	10.777cd	5.906c	5.454ef	5.637de	10.792de	4.803de	5.805e	2.399f
'Amish pie'	-	7.006de	5.782c	8.234ed	2.524e	7.412fg	6.232de	7.052de	2.606f
'Big Max'	-	4.516de	3.826c	4.770ef	2.249e	5.097fg	3.615e	4.679e	2.937ef
'Connecticut Field'	0.000d	3.199e	2.510c	4.224ef	3.170e	3.525g	3.090e	4.101e	2.641f
'Cheyenne'	-	2.397e	1.763c	2.192f	1.405e	3.872g	2.976e	3.445e	2.728f
'Cornfield'	-	3.294e	2.290c	2.726f	3.612e	3.115g	2.097e	2.810e	2.410f
'Cushaw'	-	4.828de	3.520c	5.720ef	4.420de	8.700ef	3.311e	5.943e	4.008ef
$LSD_{0.05}$:		0.251				0.298			

[1] year, season and planting date interaction was significant.
[2] means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).
[3] not grown.
[4] to compare means of same cultivar grown on different planting dates.

Data obtained for germplasm of *maxima, mixta and pepo* pumpkins production when planted on March, April, Aug., and Sept., showed similarity and dissimilarity to *moschata* species. Like *moschata*, the Egyptian landrace 'Kafr El-Battikh-2' and the exotic cvs 'Big Max' and 'Amish pie' of *C. maxima*, exotic cvs 'Cheyenne' and 'Connecticut Field' of *C. pepo* and exotic cv 'Cushaw' of *C. mixta* had the highest sugar contents in the leaves of 30-day-old plants and produced their highest fruit yield when planted on Aug., followed by planting on March. However, the Egyptian landraces 'El-Zarka' and 'Faraskour' (*C. maxima*) gave its top yield when planted on Aug., followed by planting on Sept. Exotic cv 'Atlantic Giant' (*C. maxima*) had the highest sugar contents in the leaves of 30-day-old plants and produced their highest fruit yield when planted on March. Exotic cv

'Cornfield' (*C. pepo*) showed the highest sugar contents in the leaves of 30-day-old plants and produced their highest fruit yield when planted on Sept. followed by planting on March. In agreement with results reported in Egypt by Damarany *et al.,* (1995) and Mostafa, (2006) and in USA by Wein *et al.,* (2002), the present data confirm the prominent role of planting date along with choose of cultivar in production of pumpkins. However, our study is considered the first to evidently show that availability of certain level of reduced sugar contents is crucial during flower anthesis and fruit development. This criterion may be useful to elucidate physiological basis of pumpkin fruit yield and for conducting cultivar selection.

It is noticeable, however, that fruit yield (Table 5B) and fruit contents of both TSS (Table 7B) and dry matter (Table 8A) were at a compromise when planting on March, April, May and Aug. Apparently, the Egyptian landrace cultivars 'Kafr El-Battikh-1' and 'Kafr Saad' and the exotic cv 'Early Butternut' (all belong to *C. moschata*) were the most elite cultivar germplasm with regard to fruit yield (Table 5B) and quality (Tables 7B and 8A) along with fruit storability (Table 8B). Their fruits could be stored in normal room temperature for at least about a year, on average. Due to the succession of cool weather during development of fruits produced by pumpkins planted on Sept., this planting date gave fruit with the least contents of both TSS and dry matter along with shorter storage time.

Table 6: Fruit set percentage [(number of fruit/ number of female flowers) x 100] and number of fruits per plant produced from pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons[(1)].

Planting dates	May-01	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
Seasons	1st season	2nd season				3rd season			
Cultivars									
A- Fruit set percentage [(number of fruit/ number of female flowers) x 100]									
'Kafr El-Battikh-1'	32.5 b[(2)]	34.8 abcd	42.5 abc	38.4 bc	39.7 b	31.6 def	45.8 b	34.9 cd	34.1 bc
'Kafr Saad'	36.5 b	37.9 abc	51.9 ab	49.8 ab	41.0 b	31.3 defg	42.0 bc	37.5 cd	33.9 bc
'El-Edua'	48.6 a	36.6 abcd	54.7 a	40.2 bc	40.6 b	39.1 cde	42.9 bc	37.7 cd	32.9 bc
'Qus'	47.5 a	40.9 ab	52.7 ab	40.5 bc	36.9 b	35.2 cdef	57.4 a	37.6 cd	40.1 ab
'Early Butternut'	-[(3)]	45.0 a	38.8 bc	50.0 ab	42.0 b	51.1 ab	41.4 bc	51.9 a	46.6 a
'Kafr El-Battikh-2'	0.0 c	26.8 bcdef	31.0 cd	29.5 cde	28.6 bc	44.0 abc	60.0 a	50.0 ab	23.9 cde
'Faraskour'	0.0 c	26.2 cdef	39.2 bc	54.9 a	66.4 a	40.8 bcd	35.0 bcd	40.5 bc	28.5 bcd
'El-Zarka'	0.0 c	18.2 ef	34.6 c	40.8 bc	38.1 b	37.5 cde	45.0 d	34.0 cd	29.2 bc
'Atlantic Giant'	-	27.9 bcde	29.2 cd	21.7 de	17.3 cd	27.9 efg	31.3 cde	29.6 cde	17.7 de
'Amish pie'	-	22.5 def	43.3 abc	35.0 cd	14.6 d	24.6 fgh	37.5 bcd	26.3 def	15.5 e
'Big Max'	-	22.5 def	30.8 cd	23.3 de	14.6 d	24.6 fgh	29.2 de	21.3 ef	15.5 e
'Connecticut Field'	0.0 c	12.7 f	18.8 de	20.6 e	20.8 cd	15.4 h	22.5 e	18.3 ef	14.0 e
'Cheyenne'	-	18.3 ef	23.3 e	28.8 cde	14.1 d	20.0 gh	22.5 e	16.9 f	12.2 e
'Cornfield'	-	17.5 ef	20.0 de	16.1 e	22.2 cd	20.0 gh	31.3 cde	20.8 ef	18.1 de
'Cushaw'	-	45.8 a	30.2 cd	40.7 bc	35.5 b	53.8 a	37.1 bcd	41.3 abc	36.7 ab
$LSD_{0.05}$:[(4)]		3.2				2.8			
B- Number of fruits per plant									
'Kafr El-Battikh-1'	3.0 c	4.0 cd	4.3 b	5.0 bcd	6.5 b	3.3 cd	3.8 bc	4.0 c	5.0 c
'Kafr Saad'	3.0 c	3.8 d	4.8 ab	5.5 bc	5.8 bc	2.8 de	3.0 d	3.5 c	4.3 d
'El-Edua'	4.0 b	3.8 d	5.0 ab	4.5 cd	5.8 bc	3.5 c	3.3 cd	3.8 c	4.3 d
'Qus'	4.5 a	5.0 b	5.5 a	5.8 b	6.8 b	3.8 c	5.0 a	4.8 b	6.5 b
'Early Butternut'	-	6.0 a	4.5 ab	7.0 a	8.3 a	6.3 a	4.3 b	7.3 a	8.3 a
'Kafr El-Battikh-2'	0.0 d	2.0 e	2.0 cde	2.5 e	3.0 de	2.8 de	3.0 d	3.8 c	2.3 e
'Faraskour'	0.0 d	1.8 ef	2.3 cd	4.3 d	6.5 b	2.3 ef	1.8 ef	2.8 d	2.5 e
'El-Zarka'	0.0 d	1.0 f	1.8 cde	2.8 e	3.3 d	1.8 f	2.0 e	2.0 e	2.3 e
'Atlantic Giant'	-	1.0 f	1.0 e	1.0 f	1.0 g	1.0 g	1.0 f	1.3 f	1.0 f
'Amish pie'	-	1.0 f	1.8 cde	2.0 ef	1.0 g	1.0 g	1.3 f	1.3 f	1.0 f
'Big Max'	-	1.0 f	1.3 de	1.3 f	1.0 g	1.0 g	1.0 f	1.0 f	1.0 f
'Connecticut Field'	0.0 d	1.0 f	1.3 de	1.8 ef	2.3 ef	1.0 g	1.3 f	1.5 ef	1.5 f
'Cheyenne'	-	1.0 f	1.3 de	1.3 f	1.0 g	1.0 g	1.0 f	1.0 f	1.0 f
'Cornfield'	-	1.0 f	1.0 e	1.0 f	2.0 f	1.0 g	1.3 f	1.3 f	1.5 f
'Cushaw'	-	4.8 bc	2.8 c	4.8 bcd	5.3 c	4.8 b	2.8 d	4.3 bc	4.8 cd
$LSD_{0.05}$:		0.3				0.2			

[(1)] year, season and planting date interaction was significant.
[(2)] means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).
[(3)] not grown.
[(4)] to compare means of same cultivar grown on different planting dates.

Random Amplified Polymorphic DNA (RAPD) Analysis:

Morphological features are traditionally used to assess genetic variation in *Cucurbita* species. However, many cases are controlled by quantitative factors and/or affected by environmental modification Dijkhuizen *et al.,* (1996). Recently, random amplified polymorphic DNA (RAPD) markers have been used in several studies

regarding genetic variability in pumpkin (Baranek *et al.,* 2000; Ferriol *et al.,* 2003 and 2004; Heikal *et al.,* 2008 and Athanasios *et al.,* 2009). This is probably due to the fact that it is one of the simplest and fastest molecular techniques for matching genetic similarity (Williams *et al.,* 1990).

In the present investigation, six random 10-mer primers (OPA04, OPA05, OPA06, OPA07, OPZ15 and OPB05) (Table 2 and Fig. 2) produced a total of 65 DNA bands ranged from 1365 bp (OPA06) to 530 bp (OPA04) for the three tested cultivars with an average of 10.83 bands per primer (Table 9 and Fig. 2). The highest number of amplified DNA fragments was detected using the primers OPA06 and OPA07 (13 bands). The lowest number was amplified with the primer OPB05 (8 bands). The cultivar Kafr El-Battikh, displayed the highest number of DNA fragments (59 bands). These variation in the number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome (Kernodle *et al.,* 1993). Out of the 6 primers surveyed, 5 primers detected polymorphism among the three pumpkin cultivars, while the OPB05 primer displayed monomorphic patterns. Out of 65 DNA-bands, 45 were conserved among three cultivars while 20 (30.77%) were polymorphic (Table 10).

Table 7: Average fruit weight (kg) and percentage of total soluble solids (TSS %) in fruit flesh produced from pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons[1].

Planting dates	May-01	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
Seasons	1st season	2nd season				3rd season			
Cultivars									
A- Average fruit weight (kg)									
'Kafr El-Battikh-1'	8.306 a[2]	11.126 a	8.678 a	10.792 a	6.335 a	11.014 a	8.506 a	9.206 a	6.095 a
'Kafr Saad'	6.686 b	10.011 bc	7.614 b	9.218 b	5.558 ab	10.148 ab	8.306 a	9.329 a	6.063 a
'El-Edua'	5.109 c	9.456 c	6.291 c	7.945 c	5.207 b	9.842 bc	7.402 a	8.759 a	5.815 ab
'Qus'	6.156 b	9.574 c	7.218 b	8.787 bc	5.701 ab	8.833 c	5.415 b	7.355 b	4.318 cd
'Early Butternut'	-[3]	0.421 h	0.394 i	0.287 i	0.251 g	0.544 i	0.500 g	0.326 h	0.175 i
'Kafr El-Battikh-2'	0.000 d	7.722 d	4.413 d	6.725 d	3.682 c	7.486 d	5.429 b	5.671 cd	4.720 bc
'Faraskour'	0.000 d	5.200 f	3.666 de	4.559 f	2.676 d	6.695 de	5.087 b	5.942 c	4.809 bc
'El-Zarka'	0.000 d	6.716 e	3.289 e	4.090 f	2.651 d	6.272 e	4.502 bc	5.599 cd	3.287 de
'Atlantic Giant'	-	10.777 ab	5.906 c	5.454 e	5.637 ab	10.792 ab	4.803 b	5.020 cd	2.399 efg
'Amish pie'	-	7.006 de	3.355 e	4.281 f	2.524 d	7.412 d	4.957 b	5.828 cd	2.606 efg
'Big Max'	-	4.516 f	3.108 ef	3.858 f	2.249 de	5.097 f	3.615 cd	4.679 d	2.937 ef
'Connecticut Field'	0.000 d	3.199 g	2.105 gh	2.618 g	1.537 ef	3.525 g	2.585 de	2.897 ef	1.934 fgh
'Cheyenne'	-	2.397 g	1.481 gh	1.841 gh	1.405 ef	3.872 g	2.976 d	3.445 e	2.728 efg
'Cornfield'	-	3.294 g	2.290 fg	2.726 g	1.806 de	3.115 g	1.845 ef	2.294 fg	1.597 gh
'Cushaw'	-	0.987 h	1.281 h	1.223 h	0.831 fg	1.829 h	1.200 fg	1.410 g	0.844 hi
$LSD_{0.05}$:[4]		0.239				0.345			
B- Percentage of total soluble solids (TSS %) in fruit flesh									
'Kafr El-Battikh-1'	9.4 a	7.5 c	8.1 c	6.9 c	4.7 cd	7.7 c	8.3 b	7.1 b	4.9 d
'Kafr Saad'	8.2 b	6.7 d	7.2 d	6.2 d	4.9 c	7.0 d	7.6 c	6.5 c	5.2 c
'El-Edua'	3.7 d	3.0 h	3.3 h	2.8 ij	2.6 f	3.2 i	3.4 i	2.9 i	2.7 g
'Qus'	4.7 c	3.9 fg	4.2 fg	3.5 fgh	5.1 c	4.1 gh	4.4 fg	3.7 fg	5.4 c
'Early Butternut'	-	8.2 b	8.9 h	7.5 b	7.1 b	9.0 b	10.1 a	8.7 a	7.9 b
'Kafr El-Battikh-2'	0.0 e	3.5 g	3.8 g	3.2 hi	2.0 g	3.7 i	4.0 h	3.4 h	2.1 i
'Faraskour'	0.0 e	3.7 fg	3.9 g	3.3 gh	3.3 e	3.9 hi	4.2 gh	3.5 gh	3.5 f
'El-Zarka'	0.0 e	3.0 h	3.2 h	2.7 i	2.4 fg	3.2 i	3.4 i	2.9 i	2.5 gh
'Atlantic Giant'	-	3.0 h	3.2 h	2.7 i	2.3 fg	3.2 i	3.4 i	2.9 i	2.4 h
'Amish pie'	-	4.1 f	4.5 f	3.8 f	3.4 e	4.4 f	4.7 e	4.0 e	3.6 ef
'Big Max'	-	2.9 h	3.1 h	2.6 i	2.4 fg	3.0 i	3.3 i	2.8 i	2.6 gh
'Connecticut Field'	0.0 e	4.8 e	5.2 e	4.4 e	4.4 d	5.1 e	5.5 d	4.7 d	4.7 d
'Cheyenne'	-	4.1 f	4.4 f	3.7 fg	3.6 e	4.3 fg	4.6 ef	3.9 ef	3.8 e
'Cornfield'	-	3.6 g	3.9 g	3.3 gh	3.3 e	3.9 hi	4.2 gh	3.5 gh	3.4 f
'Cushaw'	-	8.8 a	9.7 a	8.4 a	8.1 a	9.6 a	10.3 a	8.8 a	8.5 a
$LSD_{0.05}$:		0.1				0.1			

[1] year, season and planting date interaction was significant.
[2] means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).
[3] not grown.
[4] to compare means of same cultivar grown on different planting dates.

Overall, 20 specific markers were generated among the three pumpkin cultivars, using RAPD-PCR analysis (Table 11). The number of specific bands per primer varied (2-6) with size range of the fragments (530-1240 bp). The highest numbers of specific markers were obtained with primers OPA04 and OPA07 (5 and 6 fragments, respectively). The primer OPZ15 produced the lowest number of specific bands (2 fragments). The RAPD analysis revealed that the 990 bp (OPZ15), 949 bp and 595 bp (OPA06), 952 bp, 893 bp, 800 bp and 642 bp (OPA05), 1003 bp and 530 bp (OPA04) and 1240 bp, 952 bp, 590bp and 539 bp (OPA07) DNA fragments were unique positive markers to the cultivar 'Kafr El-Battikh'. However, the 705 bp (OPZ15) and 1093 bp, 928

bp (OPA07) fragments were negative markers for this cultivar (Table 11). The cultivar 'El-Zarka' was characterized by the presence of 644 bp, 589 bp (OPA04) as positive markers and the absence of 693 bp (OPA06) and 848 bp (OPA04) as negative markers (Table 11). The tested primers did not show any specific marker for the cultivar 'Connecticut field'. Fragment number 27 at molecular size 693 bp generated by OPA06 primer and fragment number 44 at molecular size 848 bp generated by OPA04 primer appeared only in two cultivars, 'Kafr El-Battikh' and 'Connecticut field'. These fragments are considered as positive specific markers for flowering response. These results were in agreement with the general flowering responses of the studied cultivar germplasm as above demonstrated.

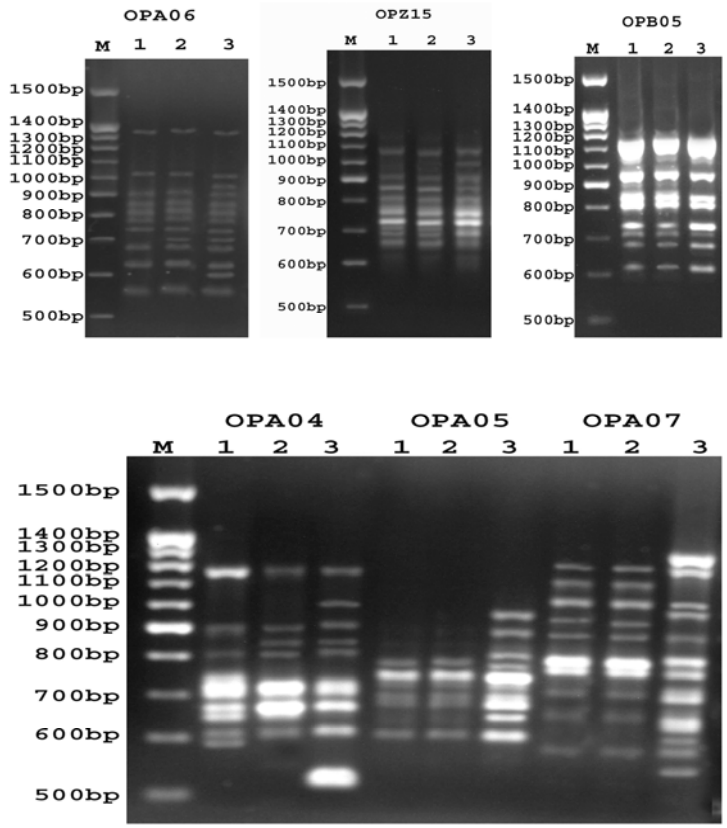

Fig. 2: Agarose gel electrophoresis of RAPD profile in the pumpkin cultivars, Kafr El-Battikh, El-Zarka and Connecticut Field cv.
1= El-Zarka 2= Connecticut field cv. 3 = Kafr El-Battikh

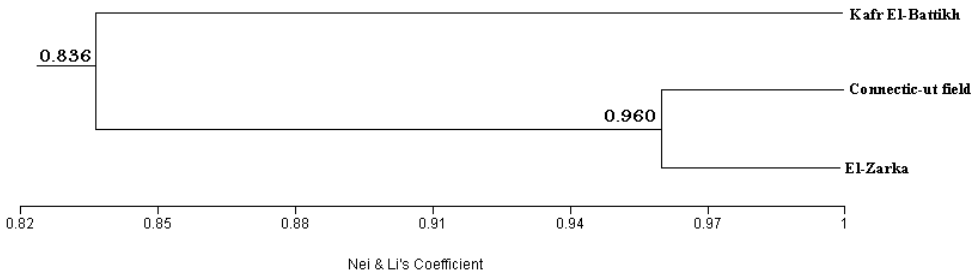

Fig. 3: Dendrogram of three pumpkin cultivars developed from RAPD data using UPGMA analysis. The scale is based on Nei and Li coefficients of similarity.

The genetic similarity among the three pumpkin cultivars was high, ranging from 81.8 % to 96 %. The highest similarity (96%) was scored between cultivars 'El-Zarka' and 'Connecticul Field' (Table 12). Meanwhile, the lowest genetic similarity (81.8 %) was found between cultivars 'Kafr El-Battikh' and 'El-Zarka' which indicated that the two lines were distantly related. Cluster dendrogram (Fig. 3) showed that the cultivar 'Kafr El-Battikh' was separated in a single branch from the other two cultivars with 83.6% branched-off genetic similarity, reflecting a relatively greater genetic distance from the other cultivars. The dendrogram also showed that both 'El-Zarka' and 'Connecticut field' were clustered together with 0.96 genetic similarity. These results obtained from RAPD analysis, in general, suggested that molecular marker approach could be considerably potential for identifying and discriminating pumpkin cultivars concern both their genetic relationship and their functional flowering response and fruiting productivity.

In conclusion, *C. moschata* pumpkins seemed to be more adapted to adverse climatic condition. Elite pumpkins may be selected towards crop improvement with aid of simple and rapid physiological and molecular techniques. Reduced sugar contents in leaves, especially during flower anthesis and fruit set and development and RAPD molecular markers seemed potential in this context. Further, this study provides the growers with useful information to decide on compromises when choosing among different planting dates and seasons for pumpkins production. Under the conditions of the present study, the Egyptian landrace cultivars of *C. moschata* ('Kafr El-Battikh-1' and 'Kafr Saad') and the exotic cv 'Early Butternut' (*C. moschata*) are generally recommended.

Table 8: Percentage of dry matter (DM %) in fruit flesh and fruit shelf life (days) for pumpkin (*Cucurbita spp.*) cultivar and Egyptian landrace germplasm grown on different planting dates in three seasons [(1)].

Planting dates / Seasons / Cultivars	May-01	Mar-01	Apr-01	Aug-01	Sep-01	Mar-01	Apr-01	Aug-01	Sep-01
	1st season	2nd season				3rd season			
A- Percentage of dry matter (DM %) in fruit flesh									
'Kafr El-Battikh-1'	10.3 a[(2)]	6.1 cd	8.7 c	8.1 c	6.9 c	6.4 d	9.7 b	8.8 b	8.0 b
'Kafr Saad'	8.7 b	6.5 cd	7.7 d	7.7 d	6.1 d	6.9 c	8.2 c	7.6 c	6.9 c
'El-Edua'	3.9 d	3.1 f	3.5 h	3.2 i	2.8 hi	3.3 g	3.7 h	3.4 h	3.1 i
'Qus'	4.9 c	6.5 c	4.4 fg	4.1 fgh	3.5 fg	6.9 c	4.7 ef	4.4 ef	3.9 fg
'Early Butternut'	-[(3)]	9.3 b	9.5 b	8.8 b	7.6 b	10.0 b	10.9 a	10.0 a	9.1 a
'Kafr El-Battikh-2'	0.0 e	2.5 g	4.1 g	3.8 h	3.2 gh	2.7 h	4.3 g	4.0 g	3.6 gh
'Faraskour'	0.0 e	4.3 e	4.2 g	3.9 gh	3.3 fg	4.5 f	4.5 fg	4.1 fg	3.8 g
'El-Zarka'	0.0 e	3.0 f	3.5 h	3.2 i	2.7 i	3.2 g	3.6 h	3.4 h	3.2 i
'Atlantic Giant'	-	2.9 fg	3.5 h	3.2 i	2.7 i	3.1 g	3.7 h	3.4 h	3.3 hi
'Amish pie'	-	4.4 e	4.7 f	4.4 f	3.7 fg	4.7 ef	5.0 e	4.6 e	4.3 e
'Big Max'	-	3.2 f	3.3 h	3.1 i	2.6 i	3.3 g	3.5 h	3.2 h	3.0 i
'Connecticut Field'	0.0 e	5.8 d	5.5 e	5.1 e	4.4 e	6.1 d	5.9 d	5.4 d	4.8 d
'Cheyenne'	-	4.6 e	4.7 f	4.3 fg	3.7 f	4.9 e	4.9 e	4.6 e	4.2 ef
'Cornfield'	-	4.2 e	4.2 g	3.9 gh	3.3 fg	4.6 ef	4.4 fg	4.1 fg	3.7 g
'Cushaw'	-	10.4 a	10.3 a	9.6 a	8.4 a	11.0 a	10.9 a	10.2 a	9.2 a
$LSD_{0.05}$:[(4)]		0.1				0.2			
B- Fruit shelf life (days)									
'Kafr El-Battikh-1'	557.4 a	333.7 b	486.9 b	314.3 b	244.2 b	364.0 b	373.9 b	242.2 b	201.2 b
'Kafr Saad'	442.6 b	330.5 b	433.8 c	279.8 c	217.3 c	349.7 c	358.5 c	205.8 c	165.5 c
'El-Edua'	221.2 d	158.2 d	195.5 e	125.8 e	97.2 e	167.3 e	206.9 e	133.1 e	109.6 e
'Qus'	270.3 c	184.4 c	246.2 d	160.5 d	124.4 d	248.9 d	263.6 d	169.8 d	139.6 d
'Early Butternut'	-	421.3 a	532.5 a	343.7 a	267.3 a	365.4 a	385.1 a	260.6 a	220.9 a
'Kafr El-Battikh-2'	0.0 e	34.2 g	43.0 h	27.7 h	21.4 gh	35.6 hi	45.5 hi	29.3 h	24.3 g
'Faraskour'	0.0 e	40.9 g	44.5 h	28.6 h	22.2 gh	43.2 hi	47.1 hi	30.3 h	25.1 g
'El-Zarka'	0.0 e	28.8 g	36.5 h	23.5 h	18.1 h	30.5 i	38.5 i	24.8 h	21.4 g
'Atlantic Giant'	-	27.8 g	36.6 h	23.5 h	18.1 h	29.4 i	38.6 i	24.8 h	21.9 g
'Amish pie'	-	42.3 g	50.0 h	32.2 h	24.9 gh	44.7 h	52.8 h	34.0 h	28.4 g
'Big Max'	-	30.0 g	34.9 h	22.5 h[h]	17.4 h	31.8 hi	36.9 i	23.7 h	20.0 g
'Connecticut Field'	0.0 e	92.4 e	97.9 f	63.1 f	48.9 f	97.6 f	103.5 f	66.7 f	53.3 f
'Cheyenne'	-	74.0 ef	82.5 fg	53.1 f	41.2 fg	78.2 g	87.3 g	56.1 fg	46.9 f
'Cornfield'	-	66.9 f	74.0 g	47.6 fg	36.9 fgh	73.0 g	78.2 g	50.4 g	41.6 f
'Cushaw'	-	35.8 g	39.1 h	25.3 h	20.0 gh	37.8 hi	41.4 hi	26.8 h	22.0 g
$LSD_{0.05}$:									

[(1)] year, season and planting date interaction was significant.

[(2)] means within column followed by same letter (s) are not significantly different at 0.05 level of probability by using Duncan Multiple Range Test (DMRT).

[(3)] not grown.

[(4)] to compare means of same cultivar grown on different planting dates.

Table 9: Survey of the RAPD-DNA fragments of the six primers in three pumpkin cultivars.

No	Primers	Bp	El-Zarka	Connectic-ut field	Kafr El-Battikh	No	Primers	bp	El-Zarka	Connecti c-ut field	Kafr El-Battikh
1	OPZ15	1066	1	1	1	32	OPA05	952	0	0	1
2		992	0	0	1	33		893	0	0	1
3		914	1	1	1	34		800	0	0	1
4		856	1	1	1	35		776	1	1	1
5		809	1	1	1	36		748	1	1	1
6		758	1	1	1	37		691	1	1	1
7		723	1	1	1	38		670	1	1	1
8		705	1	1	0	39		642	0	0	1
9		685	1	1	1	40		603	1	1	1
10		657	1	1	1	41	OPA04	1163	1	1	1
11	OPB05	1103	1	1	1	42		1003	0	0	1
12		958	1	1	1	43		905	1	1	1
13		847	1	1	1	44		848	0	1	1
14		804	1	1	1	45		807	1	1	1
15		740	1	1	1	46		715	1	1	1
16		715	1	1	1	47		669	1	1	1
17		685	1	1	1	48		644	1	0	0
18		621	1	1	1	49		610	1	1	1
19	OPA06	1365	1	1	1	50		589	1	0	0
20		1022	1	1	1	51		530	0	0	1
21		949	0	0	1	52	OPA07	1240	0	0	1
22		910	1	1	1	53		1175	1	1	1
23		857	1	1	1	54		1093	1	1	0
24		820	1	1	1	55		998	1	1	1
25		777	1	1	1	56		952	0	0	1
26		736	1	1	1	57		928	1	1	0
27		693	0	1	1	58		872	1	1	1
28		671	1	1	1	59		783	1	1	1
29		626	1	1	1	60		748	1	1	1
30		595	0	0	1	61		697	1	1	1
31		561	1	1	1	62		640	1	1	1
						63		590	0	0	1
						64		566	1	1	1
						65		539	0	0	1

Table 10: Number of amplified DNA-fragments and polymorphic bands in three pumpkin cultivars investigated with six RAPD primers.

Primers Code	No. of amplified bands			Total amplified bands	No. of polymorp hic bands	% of polymorp hic bands
	El-Zarka	Connectic-ut field	Kafr El-Battikh			
OPZ15	9	9	9	10	2	20.00
OPB05	8	8	8	8	0	0.00
OPA06	10	11	13	13	3	23.08
OPA05	5	5	9	9	4	44.44
OPA04	8	7	9	11	5	45.45
OPA07	9	9	11	13	6	46.15
Total	49	49	59	65	20	30.77

Table 11: pumpkin cultivars characterized by unique positive and/or negative RAPD markers, marker size and total number of markers.

Primer	El-Zarka		Connectic-ut field		Kafr El-Battikh		Positive marker	Negative marker	Total markers
	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker			
OPZ15	-	-	-	-	992	705	1	1	2
OPA06	-	693	-	-	949 595	-	2	1	3
OPA05	-	-	-	-	952 893 800 642	-	4	-	4
OPA04	644 589	848	-	-	1003 530	-	4	1	5
OPA07	-	-	-	-	1240 952 590 539	1093 928	4	2	6
Total	2	2	-	-	13	3	15	5	20
	4		-		16				

Table 12: Genetic similarity values calculated from 65 DNA fragments generated with six primers.

Similarity matrix	El-Zarka	Connecticut field	Kafr El-Battikh
El-Zarka	1	-----	----
Connecticut field	0.96	1	-----
Kafr El-Battikh	0.818	0.855	1

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