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## Challenges of Ongoing Cucurbits Research and Farm Gating

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Steady interest to pumpkin biology and breeding in Kazakhstan is confirmed by the former research. *Cucurbita pepo* L. is harvested throughout all the continents under different climate conditions up to 65° northern latitude. For peoples of our and neighbouring countries as populations inhabiting the Silk Road countries in general, pumpkin is one of key ingredients of sustenance. It serves as the bread dough additive and is widely used in national cuisines in the Central Asian region and beyond. Especial significance of pumpkin-based nutrition is explained by its implication in diet food and baby food due to the presence of carious form of carbohydrates, vitamins, organic acids and microelements. Abundance of nutritional composition in association with low cellulose content allows to recommend this melon crop for being included into the diet at diverse inflammation processes. Numerous medicinal applications of pumpkin is associated with cholagogue, diuretic, appetizer, anti-obesity, anti-insomnic, would-healing, baby food, diet food, anthelminthic, anti-atherosclerotic, anti-aging, anti-eczemic, cosmetic treatment and other effects. One of modern approaches in pumpkin phytoremediation is transgenic generation with programmed set of associated bacteria. This offers further transgenes for their transfer to soils, contaminating with organic pollutants. *Cucurbitaceae* spp. may be involved in postponing the aging process. Recent studies demonstrate that zucchini (*Cucurbita pepo* subsp. *pepo*) is a seasonal vegetable with high nutritional and medical values. A number of useful properties of this fruit were attributed to bioactive compounds. Earlier phylogenetic inter-relationships within the genus *Cucurbita* (pumpkins, squashes, and yellow-flowered gourds) were investigated by comparing wild and domesticated taxa. By screening an intron region from the mitochondrial *nad1* gene as a marker, taxonomic relationships in flowering plants have been clarified to show the route of domestication for *the Cucurbita* . Six or more independent domestication steps aring from distinct wild ancestors have been determined. It was proposed that *Cucurbita argyrosperma* had been domesticated from a wild Mexican gourd, *Cucurbita sororia*, growing in the Southwest Mexico, or the same region that had produced the maize. The wild ancestor of *Cucurbita moschata* remains unclear. Nonetheless, the mtDNA data has indicated that the site of origin could be located in lowland northern South America. The wild ancestor of domesticated *C. pepo* subsp. *pepo* remains unclear. However, it relates to *C. pepo* subsp. *fraterna* stemming out from Southern Mexico. One of targets for Kazakhstan is maintaining and characterizing domestic pumpkin germplasms, manufacturing the pumpkin seed oil and developing pharmaceuticals. So it is critical to study genetic power of the local accessions, expected and observed heterozygocity enhanced by intraspecious and inter-specious hybridization.

**Key words: *Cucurbita pepo* L., *Cucurbitaceae*, genetic diversity, genetic origin, squash, phytoremediation, biochemistry, medicine.**

## Современные перспективы исследования и возделывания тыквенных культур

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Устойчивый интерес к биологии и селекции тыквенных в Казахстане подтверждается предшествовавшими исследованиями. *Cucurbita pepo* L. возделывается на всех континентах, в различных климатических зонах вплоть до 65° с.ш. Для населения нашей страны и соседних государств, а также жителей стран Шелкового Пути в целом, тыква – один из основных

компонентов поддержания организма. Эта культура используется как добавка к тесту и в качестве национальных блюд в Центральной Азии и за ее пределами. Особое значение питания на основе тыквы объясняется ее применением в диетическом и детском питании, благодаря наличию легкоусвояемых углеводов, витаминов, органических кислот и микроэлементов. Богатство питательного состава в сочетании с низким содержанием целлюлозы позволяет рекомендовать эту бахчевую культуру для включения в диету при различных воспалительных процессах. Разнообразное оздоравливающее применение тыквы связано с желчегонными, диуретическими, аппетит-восстанавливающими, ранозаживляющими, антигельминтными, антисклеротическими, антиэкземными свойствами, а также способностью бороться с ожирением, бессонницей, старческими явлениями, выступать в качестве косметического средства и другими эффектами. В последнее время тыквенные также широко используются в качестве фиторемедианта. Одним из современных подходов использования тыквенных в этом направлении является получение трансгенных растений с заданным набором ассоциированных бактерий. Это позволяет получать трансгенные растения для их дальнейшего выращивания на почвах, содержащих органические загрязнители. *Cucurbitaceae* spp. могут играть благоприятную роль в процессах замедления старения. Недавние исследования показывают, что цуккини (*Cucurbita pepo* subsp. *pepo*) имеет высокую пищевую и фармацевтическую ценность. Многие полезные свойства этой культуры связаны с высоким содержанием биологически-активных веществ. Ранее путем сравнения диких и одомашенных таксонов рода *Cucurbita* (пищевые и декоративные тыквы и кабачки) проводилось изучение филогенетических взаимоотношений в пределах этого рода. Скрининг интронных областей маркерного митохондриального гена *nad1* показал таксономические связи цветковых растений, а также схему окультуривания рода *Cucurbita.* Было обнаружено не менее шести независимых этапов одомашивания дикой тыквы. Предположительно, *Cucurbita argyrosperma* происходит от дикого мексиканского вида *Cucurbita sororia*, произрастающего на Юго-Западе Мексики, в районе происхождения кукурузы. Дикий предок *Cucurbita moschata* пока не установлен. Тем не менее, база данных мтДНК мускатной тыквы показала, что место ее происхождения – низменности на севере Южной Америки. Дикий предок культурной тыквы *C. pepo* subsp. *pepo* остается неясным. Однако он является родственным разновидности *C. pepo* subsp. *fraterna,* происходящей из Южной Мексики. Для Казахстана особенно актуальным является поддержание и описание свойств отечественного генофонда тыкв, производство на их основе отечественного тыквенного масла и различных фармацевтических средств. В связи с этим, очень важно изучение генетического потенциала местных сортообразцов и форм *Cucurbita spp*, степени ожидаемой и наблюдаемой гетерозиготности, которые можно усилить за счет внутривидовых и межвидовых скрещиваний.

**Ключевые слова: *Cucurbita pepo L., Cucurbitaceae,* генетическое распространение, генетическое происхождение, длинноплодная тыква, фиторемедиация, биохимия, медицина.**

**Асқабақ дақылдарының заманауи зерттеулері және өсіру**

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Қазақстандағы асқабақтылардың селекциясы мен биологиясына аса қызығушылық бұрынғы зерттеулерге байланысты. *Cucurbita pepo L.* түрлі климаттық жағдайларда 65 ° N ендікке дейін барлық континенттерде шығарылады. Біздің еліміздің және көрші елдердің халқы үшін, сондай-ақ Жібек белдеуі елдерінің тұрғындары үшін, асқабақ - организмді сауықтырудың негізгі компоненттерінің бірі. Бұл дақыл Орталық Азиядағы және одан тыс жерлерде ұлттық тағамдар ретінде және қамырға қоспа ретінде пайдаланылады. Асқабаққа негізделген тамақтанудың ерекше маңызы оны жеңіл сіңірілетін көмірсулар, витаминдер, органикалық қышқылдар мен микроэлементтердің болуына байланысты диеталық және балалар тағамында қолдануымен түсіндіріледі. Азық құрамының байлығы целлюлозаның төмен құрамымен ұштастырылғанда, осы бақша дақылын әр түрлі қабыну процестеріне арналған диетаға қосу үшін ұсынуға болады. Асқабақты әр түрлі сауықтыруда қолдану: өт айдайтын, диуретикалық, тәбет ашатын, жара жазатын, антигельминттік, антисклероздық, антиэкземалық сияқты қасиеттеріне байланысты. Сонымен бірге, семіздікпен, ұйқысыздықпен, кәрілікпен күресу қабілеттері үшін пайдалынады. Косметикалық құралдар ретінде және басқа да әсерлері үшін қолданылады. Соңғы уақытта сондай-ақ асқабақ фиторемедиант ретінде кеңінен пайдаланылады. Осы бағытта асқабақтастарды заманауи қолдану тәсілдерінің бірі - асқынған бактериялардың жиынтығы бар трансген өсімдіктерін өндіру. Бұл органикалық ластағыштармен қаныққан топыраққа көшіру арқылы трансгендерді алуға мүмкіндік береді. *Cucurbitaceae spp.* қартаюдың баяулауына қолайлы жағдай тудыруы мүмкін. Соңғы зерттеулер көрсеткендей, цуккинидің (*Cucurbita pepo subsp.pepo*) жоғары қоректік және фармацевтикалық құндылығы бар. Бұл дақылдың көптеген пайдалы қасиеттері биологиялық белсенді заттарға байланысты. Бұрын *Cucurbita* (тамақ және сәндік асқабақ пен кәді) түріндегі филогенетикалық қатынастарды зерттеу жабайы және қолдан өсірілген таксондарды түраралық салыстыру арқылы зерттелген. Митохондрияның *nad1* маркерлі генінің интрондық аймақтарының скринингі гүлді өсімдіктерінің таксономикалық қосылыстарымен байланысын, сонымен бірге *Cucurbita* тұқымдасының қолдан өсіруге бейімдеу жолдарын көрсетті. Жабайы асқабақты қолдан өсірудің кем дегенде алты тәуелсіз кезеңі анықталды. Болжау бойынша, *Cucurbita argyrosperma* Мексиканың оңтүстік-батысында, жабайы Мексикалық *Cucurbita sororia* түрінен жүгері шыққан аймақта өскені анықталынды. *Cucurbita moschata* жабайы шыққан өсімдігі әлі анықталмады. Дегенмен, мускат асқабағының мтДНҚ дерекқоры оның шыққан жері Оңтүстік Американың солтүстігіндегі жазықтық болатындығын көрсетті. Қолдан өсірілетін *C. pepo subsp. pepo* асқабағының жабайы түрі әлі белгісіз. Алайда, Оңтүстік Мексикадан шыққан *C. pepo subsp. fraterna* түріне ұқсас.

Қазақстанның мақсаттарының бірі - отандық асқабақ генофондының қасиеттерін сақтау және сипаттау, асқабақ майын өндіру және оларға негізделген фармацевтикалық препараттарды дамыту. Сондықтан, жергілікті *Cucurbita spp.* сортүлгілерінің және түрлерінің генетикалық потенциалын, түрішілік және түраралық будандастыру арқылы күтілетін және бақыланатын гетерозигиготалық әсерлер күштерін зерттеу өте маңызды.

**Түйін сөздер: *Cucurbita pepo L., Cucurbitaceae*, генетикалық таралуы, генетикалық шығу тегі, сопақжемісті асқабақ, фиторемедиация, биохимия, медицина.**

## Historical background (Introduction)

Kazakhstan is known for steady interest to pumpkin biology and breeding. This statement may be confirmed by Dr. Taisiya G. Gutsalyuk's book "From melon to pumpkin" published in 1989. Homeland of pumpkin, *Cucurbita pepo* L. is referred to Central and South America. Cultivars of domesticated pumpkins has been recorded already 20 centuries ago. The oldest evidence, pumpkin-related seeds dating between 7000 and 5500 BC, was found in Mexico (Hartwell 2012:507). In fact, the centers of origin for the temperate species *C. pepo and C. maxima* are located throughout Mesoamerica (Decker 1988:9), whereas the subtropical *C. moschata* originated from northern Colombia (Nee 1990:49). Cucurbits have undergone several independent domestication events (Whitaker and Carter 1946:11) beginning as early as 10,000 years ago, which pre-dates maize and bean domestication (Smith 1997:932). Even such a history of early domestication witness in favour of wide distribution and utmost significance of this vegetable crop.

To date this vegetable crop is being harvested throughout all the continents under different climate conditions up to 65° northern latitude. For peoples of our and neighbouring countries as populations inhabiting the Silk Road countries in general, pumpkin is one of key ingredients of sustenance. It serves as the bred dough additive and is widely used in national cuisines in the Central

Asian region and beyond. Especial significance of pumpkin-based nutrition is explained by its implication in diet food and baby food due to the presence of carious form of carbohydrates, vitamins, organic acids and microelements. Flesh of the pumpkin fruit containing about 90% of the water is supplied with 9.7-16% of dry matter. Whereas caloricity (calorific value) of pumpkin was estimated to be 22-29 kcal, total sugar content calculated in percentage to wet matter comprises 4.8-8%, starch – 2%, cellulose (cellular tissue) – 1.0-1.2%, wet protein – 0.8-1.0%, pectin – 0.3-1.4%, fats and organic acids - 0.1% each. Some pumpkin cultivars are known to accumulate about 30% of dry matter composed by the sugar (15%), starch (23%), and ash (0.6-1.4%). However, pumpkin is ascribed to vegetable plants with lower amounts of organic acids and cellular tissues comparing to other vegetables. Abundance of nutritional composition in association with low cellulose content allows to recommend this melon crop for being included into the diet at diverse inflammation processes. Relatively low pumpkin's caloricity is the main reason for its recommendation to elderly people as the patients prone to obesity (Gutsalyuk 1989:115 and updates according to the data from www.nutrition-and-you.com/pumpkin and www.webfazenda.ru/pumpkin).

Pumpkin is distinguishable from other *Cucurbiaceae spp.* by more substantial concentrations of vitamins C (8-9 mg per 100 g of wet weight; 4-40 mg in different cultivars), B1 (thiamine, 0.05 mg per 100 g of wet weight), B2 (riboflavin, 0.06-0.11 mg per 100 g of wet weight), B5 (pantothenic acid, 0.3-0.4 mg), B6 (pyridoxine, 0.06-1.6 mg), B9 (folic acid, 14-16 μg), PP (B3, niacin equivalent, or nicotinic acid, 0.6-0.7 mg), E (tocopherol, 0.4-1.0 mg), A (carotene 250 μg, 2500-7384 IU), K (1.1 μg), B3, B5, and elatericin A (Table 1). By the carotene content pupkin is attributed to exceeding over all the vegetables and many of fruit and berry representatives. Mineral composition is presented by the following macroelements: K (204-340 mg per 100 g of wet weight), Ca (21-25 mg), Mg (12-14 mg), Na (1-4 mg), P (25-44 mg), Cl (19 mg), and S (18 mg). Mature fruits may contain nearly 30% of Cu and 0.016% of S. At the same time microelementary composition includes: Fe (0.4-0.8 mg), Zn (0.24-0.32 mg), I (1 μg), Cu (127-180 μg), Mn (0.040-0.125 mg), Co (1 μg), and F (0,86 mg). These data for American cultivars are summarized below as pumpkin (*Cucurbita spp.*) nutritive value per 100 g.

Medicinal impact of pumpkin was described by the Ancient Greek physician *Dioscorides Pedanius (*c. 40 – 90 AD*)* in his "De Materia Medica". Then Pliny the Elder (Plinius Maior, 22-79 AD), and Li Shizhen (1518 –1593) both described pumpkin's healing effects in their proceedings on medicinal plants. Pumpkin is widely used as strong cholagogue and diuretic medicines during acute and chronic nephritis, hepatitis, stomach ulcer, peptic ulcer of duodenum, against constipation and gout. As health food pumpkin is used for impaired water and salt exchange, edemas associated with heart, kidney, liver diseases or as preventive meal against atherosclerosis.Fruit flesh, seeds, stalks and flowers of pumpkin are used as medicinal raw materials. Fruit flesh is prescribed in grated as fresh forms, in form of pressed juice from raw or boiled pumpkin, as a decoction or a powder apart from a range of different dishes. The decoction of pumpkin stalks is used as the diuretic medicine, whereas the liquor made from the flowers is applied to long unhealing purulent wounds. Composition of pumpkin jouice and honey is implied as the demulcent during disturbed sleep or sleeplessness (insomnia). Fresh fruit is applied directly to the sore spot in cases of burns, skin rashes, and eczemas until full recovery.

Table 1. *Cucurbita spp*. nutritive value per 100 g.
(Source: USDA National Nutrient data base; www.nutrition-and-you.com/pumpkin)

| **Principle** | **Nutrient Value** | **Percentage of RDA** |
|---|---|---|
| Energy | 26 Kcal | 1% |
| Carbohydrates | 6.50 g | 5% |
| Protein | 1.0 g | 2% |
| Total Fat | 0.1 g | 0.5% |
| Cholesterol | 0 mg | 0% |
| Dietary Fiber | 0.5 g | 2% |
| **Vitamins** | | |

| | | |
|---|---|---|
| Folates | 16 µg | 4% |
| Niacin | 0.600 mg | 4% |
| Pantothenic acid | 0.298 mg | 6% |
| Pyridoxine | 0.061 mg | 5% |
| Riboflavin | 0.110 mg | 8.5% |
| Thiamin | 0.050 mg | 4% |
| Vitamin A | 7384 IU | 246% |
| Vitamin C | 9.0 mg | 15% |
| Vitamin E | 1.06 mg | 7% |
| Vitamin K | 1.1 mcg | 1% |
| **Electrolytes** | | |
| Sodium | 1 mg | 0.5% |
| Potassium | 340 mg | 7% |
| **Minerals** | | |
| Calcium | 21 mg | 2% |
| Copper | 0.127 mg | 14% |
| Iron | 0.80 mg | 10% |
| Magnesium | 12 mg | 3% |
| Manganese | 0.125 mg | 0.5% |
| Phosphorus | 44mg | 5% |
| Selenium | 0.3 mcg | <0.5% |
| Zinc | 0.32 mg | 3% |
| **Phyto-nutrients** | | |
| Carotene-α | 515 mcg | - |
| Carotene-ß | 3100 mcg | - |
| Crypto-xanthin-ß | 2145 mcg | - |
| Lutein-zeaxanthin | 1500 mcg | - |

Pumpkin seeds contain up to 50% of fatty oil, 45% of glycerides of linolenic acid, 25% of glycerides of oleic acid, and nearly 35% of palmitic, stearic acids, phytosterol- cucurbitol, resinous substances, organic acids, vitamins and carotenoids. This pull is successfully used in medical practice. A drug may be proposed in form of mushy substance (porridge), decoction, powder, ointment (emulsion), in natural form with sugar addition (honey-like substance or jam for better taste or appetizer). The seeds are also used as anthelmintic drug without any side effect. Treatment is usually repeated several times with 2-3 days intervals. For medical purposes the seeds are normally dried at room temperature, without over-heating. Freshly collected seeds have been recorded to possess greater healing impact than those of previous years of harvesting. For medicinal aims use of the seeds yielded over the last 2 years period is advised. The peel is detached without damage of inner gray-green seed film containing the majority of anthelminthic compounds.

By properties of the fodder pumpkin's advantages are regarded to be more valuable, if compared with other vegetables including the mangel. Pumpkin is recorded to possess 4 times more proteins as to be by 18-25% rich in respect of feed units. In addition, pumpkin is a strong milk remedy rising the milk yield and its fat content. In 2011, pumpkin was harvested from 19,140 ha in the USA. The crop was valued at the level of about $130 million (USDA, 2012). In 2010 the squash harvest including pumpkin was valued at nearly $22 million in Georgia, though pumpkins represented only 10% of the total yield (Wolfe and Luke-Morgan 2011:112). Since that time annual comparison of the farm gate value in USD has shown that over that period (2011-2016) this value for the squash has grown by 24.5% (2011 - $24,600,189; 2012 - $16,706,584; 2013 – $24,773,024; 2014 – $27,918,277; 2015 - $30,668,879; 2016 – $32,144,356). Similar farm gate value for the zucchini has also increased by 24.3%. Meanwhile, farm gate value for the wheat during the same period has dropped by 68.8% (2011 - $83,175,081; 2012 -$104,369,607; 2013 –$154,589,039; 2014 –$86,714,104; 2015 -$45,166,519;

2016 –$26,013,694) (Wolfe and Stubbs 2017:91). Finally, ornamental horticulture rised in value by $54,406,073 (+6.9%) as compared to the year 2015.

**Biological features**

Upper part as root system of pumpkin is more developed than that one of melon or water melon. Under unfavourable conditions and dry seed planting the seedlings appear in 5-8 days in form of the cotyledon leaves which are not typical of the cultivar. Type of the variety is determined by the $10^{th}$ or $15^{th}$ leaf. At this time the area of the leaf blade per plant may reach already 30-32 $m^2$. Lateral vines are formed in the sinuses of the $5^{th}$-$7^{th}$ leaves, and etc. There may be ramification (branching) of the first, second, or third order. The length of the major vine may achieve 204 m, whereas side vines may grow to 8-10 m. Total length of all the vines may encompass to 50 m.

The strength of growth and structure of roots are determined by the type of soil, its fertility, treatment, thermal conditions and moisturing, the state of subsoil horizon, level of underground water, time of sowing. Light black earth, medium loamy soils provide more extensive root development with high ramification of pumpkin plants. The taproot deepens by 1.5-2.0 m, whereas lateral roots of the first order for 2.5 m, and those of the third order – up to 1.5 m. under sufficient moisture availability pumpkin's root network develops in upper soil horizon (across 10-15 cm) by the whole width of the aisles. This requires cautious soil treatment of the aisles at the depth of 4-5 cv under standard humidity or 10 cv in case of lack of moisture. Sufficient water supply may result in additional roots formation in the internodes.

All *Cucurbitaceae* spp. are diploids with 20 pairs of chromosomes (Whitaker and Davis 1962:115). Pumpkins are a warm season annual, reaching maturity at around the fifth month. Most cultivars produce fruit on sprawling vines but bush-type hybrids are also available (Loy 2012:31). Tendrils on vines make trellising possible, but large-scale vertical production is not feasible since the large fruit require a reliable support. Broad leaves are attached to thick stems and, in most cultivars, are covered by trichomes that may occasionally cause dermatitis in case of contacting (Whitaker and Davis 1962:78).

Pumpkin is referred to monoecious plants with separated male (staminate) and female (pistillate) flowers. Pumpkins begin to proceed to anthesis in approximately 2 months after seed germination (Loy 2012:32). Adult plants produce male flowers many days prior to female flowers. As flowering continues in pumpkins for several weeks a technician or a farmer will be able to catch both a female and male flower on the same morning. Flowering and anthesis is dependent on temperature (Wien 1997:354). Then heat is used in greenhouses to increase flower development in winter. A flower is viable for a short period, just a day, remaining turgid until midday (Whitaker and Robinson 1986:219). To trigger pollination, the female flower should be bagged the evening before. Successful pollination is most likely to occur using turgid flowers in early morning hours, although if male flowers are insufficient, day-old pollen may be applied to reach appropriate fruit set. The large monoecious flowers make hand pollination suitable. So a technician may detach the desired male flower from the vine, remove the petals and thoroughly rub the large, loose pollen grains onto the entire stigma surface of the desired female flower.

Pumpkin reproduces sexually via cross-pollination (King et al. 2012:83). After 8 weeks of growth, pumpkins produce bright yellow-orange monoecious flowers that open to approximately 10 cm from an 8 cm corolla tube. The male flowers produce pollen with large granules to be transferred by the honey bee (*Apis mellifera*), bumble bee (*Bombus spp.*) and, most commonly, squash bee (*Peponapis pruinosa*) to female flowers upon pollination (Shuler et al. 2005:792). After fertilization, fruit ripens in 35-55 days after pollination depending on the cultivar (Kelley et al. 2001:112). Summer squash is harvested as immature fruit so that the soft mesocarp and seeds can be eaten entirely; winter squashes and pumpkins are harvested as mature fruit, which has a hard rind, firm mesocarp and seeds encased in a lignified seed coat attached to the fibrous endocarp adjacent to a hollow central cavity (Loy 2012:31).

## Breeding prospects

Being able to cross pollination in natural conditions, pumpkins can be bred as recommended by self-pollinating crop guidelines. This is explained by low range of inbreeding depression for most of the traits (Scott 1935:480) and flowers ability to easy pollination. In reality the breeder can maintain self-cucurbits for many generations without threat of obtaining sudden negative traits. Moreover, self-incompatibility is not recorded for the genus (Whitaker and Robinson, 1986:221). Wehner 1999:392) has estimated yield heterosis for cucurbits regarding F1 hybrids to be 40-44%. Pedigree selection is the traditional and most widespread breeding strategy since the pumpkin flower is easily hand-pollinated and generation of wide interspecific hybrids of domesticated and wild species may be successful (Loy 2012:31). Zhang et al. (Zhang et al. 2012:454) established nine interspecific bridge lines for the three domesticated cucurbit species, overcoming F1 male sterility using a number of breeding tactics. These perspective lines may be implied to various cucurbit breeding programs.

While transferring a single trait into a cultivar, i.g. specific disease resistance or plant ramification habit, backcrossing of non-elite and wild germplasm donors may turn out to be an effective strategy (Loy 2012:32). Disease resistance was also induced into commercial lines by implication of transgenic biotechnology. Transgenic lines showed higher productivity than controls even under exposition to a virus different from that one to which these lines had been insensitive (Fuchs et al. 1998:1353).

Breeders interested in quantitative traits such as fruit size, percent of dry matter or yield are encountering certain troubles as plants should be sown in huge field spaces for a nearly half-year vegetation period. So calculations and analyses should be fulfilled in different conditions as over several years. That is why conventional evaluations of available germplasm resources are rarely undertaken and reliable, replicated data on pumpkin biology and yield are deficient in the literature (Loy 2012:32).

Historically dominating open-pollinated cultivars are now being replaced by F1 hybrids (Wehner 1999:389). Hybridization of inbred lines has been a successful means for generating cultivars best suited for the edible seed and seed oil markets, where seed uniformity and seed number is selected over mesocarp characteristics (Lelley et al. 2010:479). Hand pollination makes F1 hybridization less economical for pumpkins, but modern trends in hybridization techniques may change current situation. At present, chemical induction of female (gynoecious) flowers via using ethephon is applied to *C. pepo* (Robinson et al. 1970:182) though still less effective for *C. maxima* and *C. moschata* (Wehner 1999:393). Male and bisex sterile genes have been identified in *C. pepo* and C. maxima (Carle, 1997:46; Scott and River 1946:376). However, counting present inability to phenotyping this trait during vegetation period in the field, these genes are not implied in ongoing *C. pepo* breeding programs (King et al. 2012:84). Another tool is specific method of male flowers removal from female parent plants to enable natural, bee-mediated hybridization (Curtis 1939:827) which is still too labour-consuming from point of large-scale harvesting.

## Modern research on genetic diversity of pumpkin and its relations

Significance of *Cucurbita pepo* crop has been emphasized internationally owing to its high nutritional, economic and pharmaceutical value (cited from Ntuli et al. 2015:197). The *Cucurbitaceae* family is the second most large horticultural family in terms of economic importance after *Solanaceae* (Andolfo et al. 2017:3). It includes several important crops, such as melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*) and a number of *Cucurbita* spp. with edible fruits (Jeffrey 1980:239). The genus *Cucurbita* ($2x = 2n = 40$), originated in the Americas, is composed of three economically important crop species such as *Cucurbita pepo*, *Cucurbita moschata*, and *Cucurbita maxima*, cultivated throughout sub-tropical, tropical, and temperate regions (Wang et al. 2011:218).

In South Africa leaves, flowers, fruits, seeds and shoot apices of *C. pepo* used for preparing leafy vegetable dishes It has a wide range of genetic accessions variable both in vegetative and reproductive characteristics. Small scale farmers get used to preserve available landraces by means of in situ conservation, whereas in South Africa and other countries the main tactics of preserving variability is intercropping of different Cucurbita species. Thereby the gene flow among the cucurbits is on via stochastic bee pollination. In case of gene exchange among distant plant populations new cultivars and their sub-cultivars may easily generate causing the seed exchange between farmers. Though pumpkins are naturally cross-pollinating crops, self-pollination is commonly practiced with breeding purposes. This strengthens plant homozygosity, which is not maintained by cross-pollinated species under normal conditions. This theory is confirmed by implication of molecular techniques. RAPD fingerprinting assists in determination of identical species and close genera, species-to-species gene flows, likelihood and combined genome examples, specific gene markers and mutations. RAPD markers have been used for establishing genetic diversity in cucurbits (Cuevas-Marrero and Wessel-Beaver 2008:349; Dey et al. 2006:25; Hadia et al. 2008:517; Khan et al. 2009:62; Morimoto et al. 2006:969; Tsivelikas et al. 2009:276). These markers are preferable in case of leastly known genomes and tiny DNA amounts being simple in application and producing a range of marker versions. In addition RAPD can monitor the whole genome sequence (Navajas and Fenton 2000:763; Gajera et al. 2010:496; Khan et al. 2009:64). Since recently the RAPD technique is successfully implied in South African studies (Ntuli et al. 2015:195).

Simple sequence repeat (SSR) or microsatellite markers are generated based on different eukaryotic genomes. This approach can be very informative, repetative, used under complex conditions of codominace, multiple alleles and high polymorphism. SSR technique allow to investigate slight variations across related cultivars (Formisano et al., 2012). The only limitation of this approach is availability of specific microsatellite markers (Garcia et al., 2004). Fortunately, such markers are used for Cucurbits (Formisano et al. 2012: 1179; Gong et al. 2008:42).

In Portugal, the *Cucurbita* farming is linked to conventional local cultivars and used mainly for self-consumption (human food or feed) via the sale on local markets. To evaluate the diversity of 54 *C. pepo*, 32 *C. maxima* and 21 *C. moschata* populations collected in Central and Northern Portugal, 20 morphological traits from the Minimum Descriptors for *Cucurbita* spp. and six microsatellites were used (Martins et al. 2015:68). The traits that demonstrated the maximum mean values in *C. pepo* (fruit length, skin thickness and seed weight), *C. maxima* (fruit width and 100 seed weight) in *C. moschata* (fruit weight and flesh thickness) have been determined across all three populations. The ANOVA revealed significant differences ($P<0.05$) for all traits under this study, with exception of the data on skin thickness. The Principal Component Analysis (PCA) showed that the three most informative major components explained 52.5 % of the total variation and a clear separation of the three species. The molecular analysis with SSR markers revealed a polymorphism of 100%. The observed heterozygosity (Ho) and expected heterozygosity (He) reached averagely 0.316 and 0.447, respectively. The Polymorphism Information Content (PIC) made up nearly 0.688. The hierarchical analysis revealed a clear separation of the three *Curcubita* species. The Principal Coordinates Analysis (PCoA, or classical multidimensional scaling) showed that the three most informative principal coordinates (PCs) explained 66.3 % of the total variation and clearly separated the three species under the scope. The high genetic diversity has been attributed to available Portuguese collection of *Cucurbita* spp. thus providing sufficient information on the diversity conservation and following breeding programs.

Another recent progress has been achieved by phytopathological investigations on cucurbits (Thomas et al. 2017: 6239) . In this study the focus was made on *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew (CDM) which is referred to be host-specific. This pathogen's virulence was classified into several pathotypes based on their compatibility with differential sets composed of specific cucurbit host types. Total genomic DNA was extracted from nine isolates of *P. cubensis* obtained in 2008-2013 from a number of cucurbit hosts (*Cucumis sativus, C. melo* var. *reticulatus*, *Cucurbita maxima*, *C. moschata*, *C. pepo,* and *Citrullus lanatus*) in the USA to be then subjected to whole-genome sequencing. Comparative analysis of these nine genomes revealed two distinct evolutionary lineages (lineages I and II) of *P. cubensis*. Multiple fixed polymorphisms made distinguisheable lineage I composed of the isolates from *Cucurbita pepo*, *C. moschata*, and *Citrullus lanatus* from lineage II including the isolates from *Cucumis* spp. and *Cucurbita maxima*.

Phenotypic analysis has shown that lineage II isolates were of the A1 mating type and belonged to pathotypes 1 and 3 that were not known to be present in the USA prior to the resurgence of CDM in 2004. Since the lineage II is associated with new pathotypes and lacks genetic diversity in its isolates, namely this lineage of *P. cubensis* may be provoked by the 2004 CDM resurgence in the USA. Previous studies have identified two wild cucurbit relatives, *Bryonia dioica* and *Sicyos angulatus,* as hosts compatible with both *P. humuli* and *P. cubensis* (Runge and Thines 2012:59). Susceptible varieties of hop in the United States can also be infected by *P. cubensis* (Mitchell et al. 2011:813). There could be more host types that are compatible with the two *Pseudoperonospora* species. Co-infection of different hosts may promote genetic exchange between the two species and occasional incorporation of *Pseudoperonospora* genome fragments into cucurbit genomes. Based on ongoing study, it is proposed that pumpkins hybridization may lead to the process of host specialization to *P. cubensis*. Such investigations may contribute both to identification of wild pumpkin relations and understanding true pathways of genetic exchange between crop species apart from comprehending co-infection risks and plots.

*Cucurbita pepo* includes a wide assortment of cultivars and species, known for their diverse fruit shape, color and flesh quality appreciated for essential culinary possibilities. Botanical classification based on allozyme variation recognized three subspecies in this species including: *pepo*, *ovifera* (syn. *texana*), and *fraterna*. Paris H. S. (Paris 1986:135) classified edible-fruited *C. pepo* into eight cultivar-groups: Acorn, Crookneck, Scallop, and Straightneck that belong to subsp. *ovifera* and Pumpkin, Zucchini, Cocozelle, and Vegetable Marrow that belong to subsp. *pepo* (Paris 2010:129).

The genome size of *Cucurbita* spp. is nearly 500Mb (Arumuganathan and Earle 1991:212). Recently, in addition to a draft C. pepo (subsp. pepo cultivar-group Zucchini) genome of 265 Mb was posted on CucurbiGene database completed by few *C. pepo* transcriptomes (Wyatt et al. 2015:77; Vitiello et al. 2016:318; Xanthopoulou et al. 2016:200, Xanthopoulou et al. 2017:55; Montero-Pau et al. 2017:7).

By using a high-quality zucchini (*C. pepo*) genome, Montero-Pau et al. (Montero-Pau et al. 2017:9) have shown its size of 263 Mb, a scaffold N50 of 1.8 Mb completed by 34 240 gene models. This genome is made up of 93% of the set of conserved BUSCO core genes, and it includes 20 pseudomolecules (81.4% of the assembly). The genome is associated with a genetic map of 7718 SNPs. Though the genome size is relatively small, it shows that the C. pepo genome is stemmed from a whole-genome duplication due to the following data: i, specific phylogenetic topology of the gene families; ii, distinctive karyotype pattern and distribution of 4DTv distances in couse of calculating characteristic sequence allignments; iii, assembly of 40 transcriptomes for 12 species of the genus and comparative study by using other known genomes of *Cucurbitaceae*. The duplication was detected in all the *Cucurbita* species so far investigated, including *C. maxima* and *C. moschata*. However, this duplication has not been uncovered in more distant cucurbits, i.g. the *Cucumis* and *Citrullus* genera. So such duplication, by authors' opinion, could arise 26-34 Mya in the formative ancestrial species which had served as the foundation of the genus *Cucurbita*.

Despite of that related proteome remained so far obscure. High-throughput sequencing of transcriptomes has paved new way to structural and functional information with unconceivable acceleration (Andolfo et al. 2017:3). RNA sequencing (RNA-seq) leads to extensive transcriptome generation followed by dynamic gene expression to be resulted in understanding genes function. Proteome data are further options for general genome survey and related comparative investigations. The authors sequenced and designed the first transcriptome of zucchini cv. "True French" which is resistant to pathogens and capable of producing high-grade proteome records. A total of nearly 34, 000 proteins were predicted, functionally annotated by their sequences and compared with other plant proteomes (Arabidopsis, cucumber, melon, and watermelon). Besides disease resistance gene family (R-genes) was exhaustively described as unraveled at species-specific level in *C. pepo*. The database of NCBI is comprised of preliminary sequences of three biological replicates of *Cucurbita pepo* subsp. *pepo* cultivar-group Zucchini, variety “True French”, in FASTQ format. These protein sequences of *C. pepo* can be accessed at NCBI with SAMN07426850 BioSample accession number (www.ncbi.nlm.nih.gov/Traces/study/?acc= =SRP114337). The GTF-formatted *C. pepo* transcriptome annotation and related protein seq-uences in FASTA format may be found at FIGSHARE (https://figshare. com/s/8a083f60df238acdbc19). Users can download and use the data freely for research goals with acknowledgment to the authors (Andolfo et al. 2017:5) and quoting this paper as reference to the data.

Despite crop breeding and generation of valuable traits through gene engineering (GE) is focused on specific properties, it is possible that some additional valuable features may be affected by genetic modification because of the complexity of plant metabolism and schemes of development. Recently meta-analysis profiling the phenotypic consequences of plant breeding and GE, and the comparison of modified cultivars with wild relatives in five crops of global economic and cultural importance (rice, maize, canola, sunflower, and pumpkin) has been carried out (Hernández-Terán et al 2017:7). These five species have been surveyed on phenotypic traits associated with their fitness to all the species under investigation. It has made possible to assess different processes of modification which had changed the phenotype in a definite mode through statistically reliable differences in separate phenotypic traits or specific groups of the organisms depending on their genetic origin (wild, domesticated with assistance of GE (domGE), and domesticated without involvement of GE manipulations (domNGE). The authors have concluded that genetic modification (either by selective breeding or GE) can be manifested phenotypically: i, by means of comparing domGE and domNGE with wild species; and ii, detecting the emergence and the scale of phenotypic differences between domGE and domNGE going beyond the target trait(s). This is reasonable argument as phenotypic differences of domGE and domNGE may appear to be neglectable with reference of differences between the wild and domesticated relatives (either domGE or domNGE).

The genus *Cucurbita* (pumpkins, squashes, and yellow-flowered gourds) is presented by 12–14 species scattered over the New World from the U.S. to Argentina. Earlier their phylogenetic inter-relationships were investigated by comparing six wild and six domesticated taxa of *Cucurbita* (Sanjur et al. 2002:537). By using an intron region from the mitochondrial *nad1* gene as a marker inter- and intraspecific taxonomic relationships in Angiosperms have been cleared up to yield novel insights into the route of domestication for *the Cucurbita* . Those data pointed out six or more independent domestication steps emanated from distinct wild ancestors. It was proposed that *Cucurbita argyrosperma* had been domesticated from a wild Mexican gourd, *Cucurbita sororia*, apparently in the Southwest Mexico signifying the same region that had brought maize. Though the wild ancestor of *Cucurbita moschata* remains unclear, the mitome data (mtDNA data) combined with other related information showed that the site of origin could be anticipatedly located in lowland northern South

America. In addition, it was supposed that *Cucurbita andreana*, coming from humid lowland regions of Bolivia, as the wild progenitor of *Cucurbita maxima*, apart from warmer temperate zones in South America, where *C. andreana* was initially attributed to, should possibly be considered as putative areas of origin for *C. maxima*. These as other molecular data discerned two separate domestication zones in the *Cucurbita pepo* complex. The putative domestication zone for one of the domesticated subspecies, *C. pepo* subsp. *ovifera*, was ascribed to eastern North America eventually extending to northeastern Mexico. The wild ancestor of the other domesticated subspecies, *C. pepo* subsp. *pepo*, remains unknown. However, it revealed close kinship to *C. pepo* subsp. *fraterna* which original site may locate in Southern Mexico.

## Ongoing Progress in *C. pepo* phytoremediation

Phytoremediation, or the application of higher plants for soil and groundwater recovery, is a promising strategy for reliable treatment of the lands polluted by toxic compounds. The high capability of certain Cucurbitaceae spp. to extract pesticides, namely polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) from soil was first mentioned by Hulster et al. (Hulster et al. 1994:1112) which described C. pepo L. fruits accumulating double concentrations of PCDD/PCDF comparing to other plants under that investigation. Present data demonstrate that C. pepo cultivated on contaminated soil has reduced overall PCDD/PCDF content approximately by 32-37 %. If addition of sludge has followed by root growth inhibition of *L.sativum*, *S. alba* and *S. saccharatum*, cv. 'Atena Polka' (zucchini) cultivation has caused 32 % promotion of root length. Further treatment with urban sediment resulted in an initial 1 %–36 % promotion of root growth, while 'Atena Polka' cultivation has diminished this positive effect by 26 % inhibition. So these results have indicated positive influence of *Cucurbitaceae* on the phytotoxicity abatement and decline (Urbaniak et al. 2017:403). Systemic analysis of different members of the *Cucurbitaceae* family, including cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), pumpkin (*Cucurbita pepo*), squash (*C. pepo*), and zucchini (*C. pepo*) selectively impured with highly toxic hydrophobic pollutants (organochlorine insecticides and dioxins) has been intiated quite recently by Inui et al. Xylem sap of C. pepo ssp. Pepo which is accumulating hydrophobic compounds was shown to transfer pyrene (the hydrophobic compound) into the soluble state by using proteins. SDS-PAGE of xylem sap of two *C. pepo* subspecies has determined the quantity of 17-kD proteins from C. pepo ssp. Pepo. It appeared to be greater than that from *C. pepo* ssp. ovifera. So the authors have proposed that these low molecular weight proteins may be responsible for the transfer of hydrophobic pyrene into the aqueous phase. 17 kD electrophoretic band was shown to conclude major latex-like proteins (MLPs). Relevant MLP-PG1, MLP-GR1, and MLP-GR3 genes have been cloned from the *C. pepo* cultivars "Patty Green" and "Gold Rush". Expression of the MLP-GR3 gene in C. pepo cultivars was found to positively correlate with 17-kD band thickness and bioconcentration feature to dioxin and its derivative compounds. Recombinant MLP-GR3 has been revealed to be able to bind polychlorinated biphenyls attached to magnetic beads, whereas recombinant MLP-PG1 and MLP-GR1 eluted without binding. These data demonstrate the high expression of MLP-GR3 in pumpkin (*C. pepo ssp*. pepo plants) as the efficiency of MLPGR3in the xylem sap for the translocation of hydrophobic impurities. Such data may be useful for reduced contamination of fruits across the *Cucurbitaceae* family and the phytoremediation in respect of hydrophobic contaminants. The draft genome of *Enterobacter aerogenes*, a Gram-negative bacterium of the *Enterobacteriaceae* isolated from *Cucurbita pepo* root tissue has been recently reported (Eevers, 2015a:490). This bacterium manifests 2,2-*bis*(*p*-chlorophenyl)-1,1-dichloroethylene (DDE)-degrading potential supplemented by substantial plant growth promotion. Survey on its 4.5-Mb preliminary genome sequence would enable our comprehension of DDE degradation metabolic pathways as well as application of phytoremediation to DDE-polluted soils. At the same time another genome of Gram-negative bacterium *Methylobacterium radiotolerans* was also isolated from *Cucurbita pepo* roots. This bacterium possessed also DDE-degrading potential and plant growth-promoting features. Analysis of 6.8-Mb genome of that species should also upgrade our current knowledge on DDE-transformation metabolic pathways and assist in improving phytoremediation tools to recover DDE-impured environment (Eevers, 2015b:448). This series of of experiments are being followed by publication of the draft genome for *Sphingomonas taxi* isolated from *Cucurbita pepo* root tissue. This Gram-negative microorganism also revealed DDE-degrading trait and plant growth-accelerating property. More precise investigation of composition of its 3.9-Mb preliminary genome will contribute to the data on DDE-utilization crosstalks and provide subsequent phytoremediation techniques for DDE-polluted lands (Eevers, 2015c:317). All three genomes represent a signle circular chromosome with 53.8-71.2% GC content, 3634- 6373 coding genes, arranged in 309-378 pathways, 260-1474 pseudogenes, 3-40 rRNA genes, 40-50 tRNA genes, and 1-7 noncoding RNA(ncRNA).

Another modern approach is generation of transgenic with programmed set of associated bacteria. This trend is supposed to offer further generation of genetically modified plants to be implied for environmental-friendly soil and water treatment in case of their contamination with organic substances (i.g., polychlorinated biphenyls, PCBs, Van Aken et al. 2010:771). This attitude may foster phytoremediation of PCB-polluted environment via the design of transgenic plants with certain associated microorganisms. Plants can improve the situation with environmental toxic substances imposing a number of mechanisms. In addition to direct "aspiration" from soil (*phytoextraction*), plants may exploit enzymatic modification of PCBs (*phytotransformation*). Plants are also able to synthesize and transfer a range of secondary substances or activate microorganisms inhabiting the root zone to neutralize PCBs (*rhizoremediation*). All these pathways of phytoremediation may occur too sluggish, as PCBs are hydrophobic and chemically durable. So the uptake and bioconversion by plants and associated microflora is slow and incomplete with further possibility of PCBs toxic reverse leakage to surrounding environment. It is necessary to emphasize that natural bacterial associations with plants may appear to be unable to carry out PCB detoxication. To complete their enzymatic "store" sets of bacterial genes effectively converting PCBs, and first of all, biphenyl dioxygenases, have been inserted into higher plants genome according to convenient gene engineering approaches leading to transgenic crops. Bacteria have been additionally genetically modified to reach better biodegradation outputs through effective plant-bacterial interaction before and during this process. Generation of transgenic associated with bacteria would pave way to broader and efficient implication of phytoremediation for the elimination of hazardous organic substances.

Recenly Antoine et al. (Antoine et al.2017: 185) thirteen Jamaican-grown food crops (pumpkin (*Cucurbita pepo*), ackee (*Blighia sapida*), banana (*Musa acuminate*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cassava (*Manihot esculenta*), coco (*Xanthosoma sagittifolium*), dasheen (*Colocasia esculenta*), Irish potato (*Solanum tuberosum*), sweet pepper (*Capsicum annuum*), sweet potato (*Ipomoea batatas*), tomato (*Solanum lycopersicum*) and turnip (*Brassica rapa*) were examined for aluminium, arsenic, cadmium and lead by atomic absorption spectrophotometry (AAS) and instrumental neutron activation analysis (INAA). By using the fresh weight mean concentrations in these food crops (4.25–93.12 mg/kg for aluminium, 0.001–0.104 mg/kg for arsenic, 0.015–0.420 mg/kg for cadmium, 0.003–0.100 mg/kg for lead) the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and target cancer risk (TCR) for arsenic, associated with dietary inclusion of these potentially toxic elements was defined. Each food type had a THQ and HI < 1 indicating no essential carcinogenic risk from exposure to a single or multiple potentially toxic elements from the same food. The TCR for arsenic in these foods turned out to be lower than $1\times 10^{-4}$, the upper threshold of acceptable cancer risk. It has been concluded that there would be no potential threat associated with the consumption of pumpkin and other Jamaican-grown food crops.

Current research on trace metals highlighted in recent Darwin review (Andresen et al. 2018:926) indicated their peculiarity as specific micronutrients combined with puissant toxicity. Due to environmental and anthropogenic factors, contrastly different trace metals amounts are detected in various areas, ranging from negligible to toxic contents. So modern phytoremediation makes its emphasis on plant response to trace metals at steps of uptake, transfer, capture from the environment (sequestration), speciation, physiological use, deficiency, toxicity, and their disposal (detoxification). Each of these processes of metal usage, transfer, or toxicity may be studied among pumpkin and its relations as model plants by broadening gene expression assays, biochemical and iophysical tools applied to metalloproteins. Two other reviews of the same issue of the Journal of Experimental Botany are focused on molecular effectors of fertilization-free (parthenocarpic) fruit development (Joldersma and Zhongchi Liu, 2018) and dehydration survival maintained by delayed mortality or impaired by plant metabolic resilience (Blum and Tuberosa, 2018:207). Both aspects may be successfully as certained by conducting experiments on *the Cucurbitaceae* with following practical outcome in the cropping and production.

**Present medicine as advanced application platform for Cucurbitaceae spp.**

Medicinal significance of pumpkin has been stressed in the introduction. *Cucurbita pepo*, *Amaranthus dubius*, *Vigna unguiculata* have been implied traditionally against obesity in Kenya. Recent studies have been concentrated on determining the antiobesity activity of leaf methanolic extracts from these plants in progesterone-induced mice with overweight (Nderitu et al. 2017:8). The activity of leaf extracts was identified by bioscreening in progesterone-induced obese mice at 200mg/kg and 400mg/kg. Body mass index was calculated once a week for 30 days. Blood samples were then obtained to be subject to lipid profiling. Antiobesity plant extract activities were compared to the controls. Leaf extracts of C. pepo and other plants, at above-mentioned dose concentrations revealed significant effects on body mass index. At concentration of 200 mg/kg of C. pepo extract treatment weight gain has been observed at the level of 2.7%, whereas at 400 mg/kg weight loss has been determined to be around 0.3%. No significant difference of the three plant extracts in lipid profiling has been registered. Plant extracts contained various phytochemicals such as saponins, flavonoids, alkaloids, and steroids. Thus aforementioned plants, and pumpkin among them are recommended for the suppression of obesity and its effective management.

Carotenoids possessing diverse biological effects, are able to function as antioxidants protecting eye tissues against free radicals. The exclusive source of carotenoids for humans is food, and the carotenoid abundance and availability in plasma is critical for the maintenance of tissue viability in the long run. Pumpkin, zucchini squash, and yellow squash together with some other plant and egg sources are considered as major founts of carotenoids. In the middle of 1990s it was suggested that eating these vegetables, which are rich in carotenoids, may decrease the risk of age-related macular degeneration. The goal of this study (Sommerburg et al.1998:908) was to determine those fruits and vegetables which contain abundant lutein and/or zeaxanthin to serve as putative dietary supplements for these carotenoids. Homogenates of 30 fruits and vegetables, two fruit juices, and egg yolk, completed by homogenized pumpkin, zucchini squash, and yellow squash fleshes, were used for extracting the carotenoids with hexane. Different factions of carotenoids and their isomers were measured by high performance liquid chromatography with a single column eluted in an isocratic mode and supplied with a diode array detector. Though the egg yolk and maize (corn) revealed the highest mole percentage of lutein and zeaxanthin (more than 85% of the total carotenoids), substantial quantities of lutein and zeaxanthin (30–50%) were also present in different kinds of squash, zucchini (vegetable marrow), kiwi fruit, grapes, spinach, and orange juice. Maize was referred to the vegetables with the largest amount of lutein (60% of total), whereas orange pepper was the vegetable with the highest quantity of zeaxanthin (37% of total). The results show that there are squashes as other fruits and vegetables of various colours with a relatively high content of carotenoids. The conclusion has been made that most of the dark green leafy vegetables, previously recommended for a higher intake of lutein and zeaxanthin, possessed 15–47% of lutein, but a very insignificant amount (0–3%) of zeaxanthin. So this study advises squashes as other fruits and vegetables of various colours to enrich dietary intake of lutein and zeaxanthin. Taking into consideration a fivefold higher concentration of lutein and zeaxanthine in the macula compared with the peripheral retina, such strategy of food consumption or dietary supplementation may lead to prevention of macular degeneration in our conditions and worldwide.

Growing demand, production and consumption of natural drugs and their pharmaceutical applications has promoted a worldwide research on medicinal plants serving as effective remedies. One of prospective goals still underestimated in Kazakhstan (the authors have submitted two projects encouraged by foreign experts but finally declined for state grant financement) is the seed oil of pumpkin (*Cucurbita pepo* L.). This oil is ascribed to possess outstanding pharmacological features, especially with reference of wound healing treatments (Bardaa et al. 2016:9). The content and composition of certain bioactive ingredients of the pumpkin seed oil obtained by cold pressing were established to study their wound recovering properties. Uniform injuries were induced on the backs of eighteen rats. This cohort was divided into three groups. The wounds were registered by photography, and treated with saline solution (0.9% NaCl, control group), 0.13 mg/mm2 of a reference commercial drug ("Cicaflora cream"), and 0.52 µl/mm2 of pumpkin's oil every 48 hours until the first group has been completely recovered and biopsies would be histologically assessed. The content and composition of tocopherols, fatty acids, and phytosterols were shown to reveal perfect quality of pumpkin oil concluding a high content of polyunsaturated fatty acids (linoleic acid, $50.88 \pm 0.11$ g/100 g of total fatty acids), tocopherols (280 ppm) and sterols ($2086.5 \pm 19.1$ ppm). Increased amount of these bioactive ingredients has caused effective wound healing confirmed in course of this in vivo experiment. Morphometric evaluation and histological analysis revealed healed biopsies posteriously to the pumpkin oil treatment of experimental group of rats, and a complete re-epithelialization with re-organized collagen fibers without inflammatory cells, whereas control group remained obviously injured. So this in vivo study emphasized the significance of the seed pumpkin oil as a potential drug to healing human and animal injuries. Moreover, certain efforts should be taken towards introducing tocopherols, sterols and polyunsaturated fatty acids of the pumpkin oil as excellent drugs and ingredients to be used in cosmetics. In general, seed pumpkin oil is recommended for the nutritional and medicinal purposes. On the other hand, a number of investigators have reported growing susceptibility to nematodes in both humans and livestock. Grzybek et al. (Grzybek et al. 2016:15) conducted a special study to assess the in vitro and in vivo anthelmintic effect of *Curcubita pepo* L. For this aim hot water extract (HWE), cold water extract (CWE) and ethanol extract (ETE) have been examined on two model species of nematodes, *Caenorhabditis elegans* and *Heligmosoides bakeri*. Raman, IR and LC-MS spectroscopy analyses were carried out on plant material under investigation to collect qualitative and quantitative data on the composition of the obtained HWE, CWE and ETE extracts. The in vitro activity has demonstrated the impacts of C. pepo extracts on *C. elegans* and various developmental stages of *H. bakeri*. The following in vivo experiments on mice infected with *H. bakeri* confirmed inhibitory properties of the ETE as the most active pumpkin extract selected by the in vitro study. All of the extracts were detected to contain cucurbitine, aminoacids, fatty acids, and berberine and palmatine. The latter two substances were revealed for the first time. All *C. pepo* seed extracts showed a nematidicidal property in vitro, specifically affecting viability of the larvae, L1 and L2, of H. bakeri. The ETE was the strongest and demonstrated a positive effect on H. bakeri oviposition (eggs hatching) and marked inhibitory effect on the worm motility comparing to a PBS-buffered control. No significant effects of pumpkin seed extracts on *C. elegans* integrity or maneuverability were indicated. The ETE in the in vivo experiments manifested anthelmintic properties while both H. bakeri fecal egg counting and adult worm burdens calculating. The most substantial egg number reduction was observed at the concentration of 8 g/kg dose (IC50 against H. bakeri = 2.43; 95% Cl = 2.01–2.94). Dropping of faecal egg counts (FEC) was accompanied by a significantly lowered worm burdens of the treated mice compared to the control cohort. Thus the alcohol and the water pumpkin seed extracts may be used to administrate gastrointestinal (G.I.) nematode infections. This cheap alternative to the currently available chemotherapeutic should be regarded as a powerful curative candidate in the short run. The researchers predict new extensive methods for extract stabilization, preservation, and formulation.

Noteworthy, long tradition of pumpkin seed oil production belongs to Austria, Croatia and some other countries. Recent chemical study allowed to clarify antioxidant behaviour of the pumpkin seed oil or mixtures owing to implication of six mathematical models (three monophasic and three biphasic) implied (Broznić et al. 2016:205). The used oil samples differed in methods of extraction as geographic origin. Possible participation of tocopherol isomers (α-, γ- and δ-) and specific fatty acids (oleic and linoleic) in DPPH˙ disappearance (2,2-diphenyl-1-picrylhydrazyl radical disappearance) as well as their contribution to the reaction rate were estimated. These experiments have revealed that γ- and δ-tocopherols prevented from DPPH˙ disappearance in the first step,whereas α-tocopherol in the second step of the reaction. In addition α-tocopherol has shown 30 times higher antioxidant activity, when compared with γ- and δ-tocopherols. So the two-phase double-exponential manner of DPPH˙ disappearance in oil samples has been determined , due to the complex reactions encompassing various tocopherols and passing through alternative chemical pathways. Thus, the authors recommend the use of a first-order double-exponential model to determine biphasic DPPH˙ disappearance which offer prompt and precise information on antioxidant potential in respct of the differences in oil's chemical composition. This model proposes that the oil tocopherol isomers possess different radical suppressing ability. These isomers then react with radicals at different rates, thereby assigning a two-step reaction. For a deeper comprehension of the mechanisms for the DPPH˙ and oil antioxidants participation, it is high time to give detailed description of the pumpkin oil with regard to specific antioxidants, the reaction intermediate products and also those products which reveal different binding or other activities to the radicals. New mathematical models combining all the properties and special cases have been forecasted by this group of chemists.

Do *Cucurbitaceae* spp. may be involved in controlling the process of aging? Recent study reported by Martínez-Valdivieso et al. (Martínez-Valdivieso et al. 2017:17) points out that zucchini

(*Cucurbita pepo* subsp. *pepo*) is a seasonal vegetable with high nutritional and medical values. A number of useful properties of this fruit were attributed to bioactive compounds. Zucchini fruits (cvs. “Yellow” and “Light Green”) and four distinctive components (lutein, beta-carotene, zeaxanthin and dehydroascorbic acid) were chosen for tracing. Initially, the lutein, beta-carotene, zeaxanthin and dehydroascorbic acid concentrations were detected in fruits of these cultivars. To assess the safety and suitability of fruits, different assays were performed: (i) genotoxicity and anti-genotoxicity tests to indicate the safety and DNA-protective features to hydrogen peroxide; (ii) cytotoxicity; (iii) DNA cleavage (fragmentation) and Annexin V/PI (Propidium Iodide) assays to ascertain the pro-apoptotic effect. These experiments showed that: (i) all the substances were non-genotoxic; (ii) moreover, these substances revealed anti-genotoxicity except high lutein concentrations; (iii) “Yellow” zucchini epicarp and mesocarp were detected to possess the maximum cytotoxic index (IC50 > 0.1 mg/ml and 0.2 mg/ml, respectively); and (iv) “Light Green” zucchini skin enzymes induced inter-nucleosomal DNA fragmentation, and beta-carotene served as the putative molecule evoking pro-apoptotic activity. So zucchini fruit could positively effect human health and nutrition, since its components were found to be safe, able to hinder substantially the H2O2-induced damage and demonstrate anti-proliferative and pro-apoptotic activities among tumor cells (namely HL60 human promyelocytic leukemia cells). Valuable information generated by this study should be taken into consideration while selecting potential resources for Cucurbitae breeding programmes and designing anti-aging programmes for humans, livestock and other animals of interest.

**Current trends in pumpkin biochemical research**

Though the fruit mesocarp and the seeds of pumpkins and winter squashes are used for consumption, the focus of breeding depends on available stock of cultivars. Cultivars aimed at fruit consumption are bred for fruit mesocarp quality traits including carotenoid and dry matter abundance, percentage of soluble substances solids. These important traits are less critical while evaluating oilseed pumpkins (Wyatt et al. 2016:7). To assess fruit development in two types of squash (acorn squash and oilseed pumpkin), the researchers sequenced the fruit transcriptome of two cultivars bred for different purposes: an acorn squash (cv. “Sweet REBA”), and an oilseed pumpkin (cv. “Lady Godiva”). Putative metabolic pathways were designed for starch, sucrose and carotenoid syntheses in winter squash and squash fruit homologs with the identification of related sets of structural genes along these pathways. Gene expression, including branch-point and rate-limiting genes, has shown high matching with the accumulation of metabolic products both in course of developmental stages and between the two cultivars. So developmental control of metabolite-converting genes is a crucial factor in cucurbits fruit and, possibly, oil quality.

Phenolic compounds were extracted from pumpkin (*Cucurbita pepo*) seed and amaranth (*Amaranthus caudatus*) grain using 80% (v/v) methanol (Peiretti 2017:2179). Both extracts were characterized by total phenolic compounds (TPC) composition, trolox equivalent antioxidant capacity (TEAC), ferric-reducing antioxidant power (FRAP) and antiradical activity to specif radical (2,2-diphenyl-1-picrylhydrazyl, or DPPH$^{\cdot}$). By HPLC-DAD technique concentrations of separate phenolic compounds was detected. Pumpkin seeds showed higher content of TPC in comparison to that from amaranth, although the TEAC values of both extracts were similar. Oppositely, FRAP value was determined to be higher in the amaranth grain comparing to the pumpkin seed. Phenolic composition of the amaranth grain indicated strongest antiradical activity to DPPH radical. HPLC charts of both extracts demonstrated several peak fractions. The UV-DAD spectra revealed derivatives of vanillic acid in the amaranth grain. Simultaneously, the three main phenolic compound were shown to be present in pumpkin seed by UV-DAD spectra with maximums at 258, 266 and 278 nm.

*Cucurbita pepo* is used in folk medicine against gastroenteritis, hepatorenal and brain abnomalies. *Cucurbita pepo* fruit skin (peel) has been noticed to possess protective effect in rats against carbon tetrachloride-induced (CCl4-induced) neurotoxicity (Zaib and Khan 2014:1969). In that study, 36 Sprague-Dawley female rats (190±15 g) were subdivided into 6 groups of 6 rats each. Group I was given 1 ml/kg bw (body weight) of corn oil intraperitoneally (i.p.); Group II- IV were treated with 20% $CCl_4$ dispersed in corn oil (1ml/kg bw i.p.). In addition Group III and IV animals were treated with CPME (the methanol extract of *C. pepo* fruit peel) at 200 and 400mg/kg bw respectively. Animals of Group V and VI were injected only with CPME at 200 and 400mg/kg bw respectively. All treatments were administered for 3 days a week for a fortnight. $CCl_4$ injection caused acute neurotoxic reaction traceable by significant decrease of antioxidant enzymes activities (catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, peroxidase, quinone reductase, and superoxide dismutase). On the other hand, $CCl_4$ treatment rised the γ-glutamyl transferase activity of brain samples. $CCl_4$ intoxication decreased the reduced glutathione (GSH) content combined with significantly growing lipid peroxidation in the same samples. Co-treatment of CPME obviously protected the brain tissues against $CCl_4$-indiced damages by recovered antioxidant enzymes and strengthened lipid peroxidation in a dose dependent mode. Such neuroprotective changes might be explained by available antioxidant components.

Defatted oil cakes are used in the production of protein concentrate and isolate. Protein extraction is carried out to perform further protein purification and concentration. So extraction with different solvents is a principal operation in manufacturing a range of protein preparations (Gia Loi Tu et al., 2015:485). Ultrasound- and enzyme-assisted extractions of albumin (water-soluble protein group) from defatted pumpkin (*Cucurbita pepo*) seed powder were subjected to comparative analysis. These modern extraction techniques have strongly magnified the yield of albumin faction, if compared with conventional extraction technique. One of the advanced methods, the ultrasound-assisted technique has appeared to reveal two times higher extraction rate than that of the enzymatic-assisted extraction. However, the maximum albumin yield obtained by enzymatic albumin purification has been 16 % higher than ultrasonic extraction. Obtained pumpkin seed albumin concentrates have been studied by the Vietnamese scientists for their functional properties. This became possible due to application of conventional, enzymatic, and ultrasonic methods of evaluation. Cell wall distruction by the hydrolase digestion of the plant material has not influenced the functional properties of the albumin concentrate, when compared with the conventional way of extracting. The ultrasonic extraction enhanced water-retention, oil-retention and emulsifying capacities of the seed albumin concentrate. Simultaneously, this method has been shown to imperceptibly decrease the foaming capacity, besides emulsion and foam stabilities.

Cadmium (Cd) stress may be diminished by means of the phytochelatins (Pc) complexation, binding of the metal to metallothioneins and glutathione, followed by their sequestration inside of vacuoles (Kolb et al. 2010:92). Glutathione was proven to be a major party in Cd detoxification. This way is possible due to Cd ability to bind with a high affinity to thiols together with glutathione's ability to function as the precursor for Pc synthesis. In this study, the Austrian team has implied an immunohistochemical assay combined with transmission electron microscopy. This method has allowed to assess quantitatively intracellular distribution of glutathione under Cd stress in cells of the mesophyll and different types (long and short stalked) of glandular trichomes isolated from *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB. No ultrastructural alterations were found in leaf and glandular trichome cells upon plant treatment with 50 μM cadmium chloride ($CdCl_2$) for 48 hours. However, all cells exhibited reduced glutathione concentrations. The strongest reduction was shown in nuclei and the cytosol (up to 76%) of glandular trichomes to be considered as 'major storing cells of leaf Cd accumulation. The glutathione ratio between the nuclei and the cytosol and the rest of the cell compartments was shown to severely drop only in glandular trichomes (over 50%). This has depicted that nuclear and cytosolic glutathione is crucial for the detoxification of Cd in glandular trichomes. Moreover, these research has demonstrated that large portion of Cd is released from nuclei in case of Cd exposure. So these data provide a new comprehension on organelle-specific biochemical role of glutathione under Cd exposure happening to mesophyll cells and glandular trichomes of *C. pepo* plants.

**Conclusion**

This review demonstrated molecular data indicating two separate regions of domestication for the *Cucurbita pepo* complex. One of such zones (*C. pepo* subsp. *ovifera),* was attributed to Eastern part North America stretching out to Northeastern Mexico. The wild ancestor of domesticated *C. pepo* subsp. *pepo* remains unclear. However, it relates to *C. pepo* subsp. *fraterna* stemming out from Southern Mexico. Current research has shown that C. pepo grown on contaminated soil is able to lower total polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) contents by 30-40%. In addition C. pepo cultivation on organically impured soil may lead to 32% of root extention. So there is positive effect of *Cucurbitaceae* on soil phytotoxicity and subsequent recovery (Urbaniak et al. 2017:405). Multiple medicinal potential of pumpkin is associated with cholagogue, diuretic, appetizer, anti-obesity, anti-insomnic, would-healing, baby food, diet food, anthelminthic, anti-atherosclerotic, anti-aging, anti-eczemic, cosmetic treatment and other effects. Seeds and fruit organs of pumpkin are used as medicinal raw materials. Fruit mesocarp, pressed juice, decoctions, powders and liquors made of pumpkin stalks and flowers are widely used to long unhealing wounds, insomnia and other disturbances.

Over the past decade, efficient genetic transformation tools have been offered for pumpkins and squashes. One of such methods has been proposed for kabocha squash (*Cucurbita moschata* Duch. cv. Heiankogiku) based on wounding of cotyledonary node explants with aluminum borate whiskers preceding microbial inoculation with *Agrobacterium (*Nanasato et al. 2011:1461). Steps of transformation of *C. moschata* plants included seed germination, obtaining cotyledonary node explants with overnight dark pre-cultivation, stirring with aluminium whiskers, *Agrobacterium* inoculation, shoot regeneration and kanamycin incubation, shoot-root induction and elongation, selection of true (non-chimeric) transgenes by auxiliary bud induction and transplantation to the soil. Adventitious shoots were induced exclusively from the proximal regions of the cotyledonary nodes by cultivating on Murashige–Skoog agar medium with 1 mg/l benzyladenine. Stirring with 1% (w/v) aluminum borate whiskers promoted the *Agrobacterium* infection in the proximal zones of the explants. Transgenes were selected at the T0 generation by sGFP fluorescence, genomic PCR, and Southern blot analyses. These transgenes were shown to grow conditionally until T1 seeds formation and maturation. The authors revealed steady transduction of transgene’s inheritance to T1 generation by sGFP fluorescence and genomic PCR analyses. The average transgenic efficiency for the kabocha squashes has achieved approximately 2.7%, admissible for practical implementation. This approach is useful for practical needs as gene engineered *Cucurbita spp.* have been delayed by difficulties in genetic transformation. Nanasato et al. *(*Nanasato et al. 2011:1465). have determined tools for molecular breeding of the *Cucurbita* genera.

One of perspective purposes for Kazakhstan is to maintain and characterize domestic collection of pumpkins, produce the pumpkin seed oil and develop pharmaceuticals to be exported as used for medical and food-supplying aims. To develop domestic collection of productive cultivars of mutiseed oilseed pumpkins with susutainable yield we have initiated pilot experiments in 2014 . (Nurzhanova et al., 2014). To date three cultivars of oilseed pumpkin from Austria, Russia and China are available. In the future we hope to generate and submit to state trial highly productive cultivars and lines of pumpkins with essential oil content, fruit quality, resistance to a number of diseases as abiotic environmental factors. These forms will be subdivided by fruit size, vine branchin and the quality of oil-containing seeds.

From theoretical point, it is critical to study genetic power of the local pumpkin accessions, determine their expected and observed heterozygocity enhanced by intraspecious and inter-specious hybridization in conditions of the South-East of Kazakhstan and wider.

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