Review paper

Shallots in Croatia – genetics, morphology and nomenclature

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Abstract – The term 'shallot' in Croatia denotes three genetically and morphologically different, vegetatively reproduced relatives of the common onion, *Allium cepa* L., which are mainly traditionally cultivated for consumption and as a spice: *A. cepa* Aggregatum group, ($2n = 2x = 16$), *A. × proliferum* (Moench) Schrad. ($2n = 2x = 16$) and *A. × cornutum* Clementi ex Vis. ($2n = 3x = 24$). This paper reviews the results of studies of their morpho-anatomical characteristics and genetic structure. Although all three taxa were determined as varieties of the common onion, only the shallot *A. cepa* Aggregatum group (syn. *A. ascalonicum* L.) belongs to that species. The shallot *A. × proliferum* represents a hybrid between the two closely related species, *A. cepa* and *A. fistulosum* L. The third form of shallot, *A. × cornutum* is a still incompletely understood triploid hybrid between *A. cepa* and one or two closely related *Allium* species, whose identity has not been fully elucidated. In contrast to shallot *A. cepa* Aggregatum group, which has normal meiosis and produces fertile seed, hybrid shallots *A. × proliferum* and *A. × cornutum* are sterile, and reproduce exclusively vegetatively by underground bulbs or bulbils from the inflorescence.

Key words: *Allium cepa*, shallot, hybrid, polyploidy, triploid viviparous onion, top onion, karyotype, genome, meiosis

Common onion, *Allium cepa* L.

The genus *Allium* comprises about 750 species distributed all over the northern hemisphere except for its tropical regions (STEARN 1992, FRITCH and FRIESEN 2002). The common onion is the most widespread and economically important species of the genus *Allium* (HANELT et al. 1992, FRITCH and FRIESEN 2002). Taxonomically, it belongs to *Allium* section *Cepa* (Mill.) Prokh, which consists of twelve species, most of which are used as condiment, vegetable or medicinal plants (GURUSHIDZE et al. 2007).

The common onion is not known in the wild form. Its initial domestication started more than 4000 years ago, probably in the Middle East (HANELT et al. 1992). Several wild relatives of the common onion originate from the mountainous regions of Asia in the region be-

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tween South Siberia, the Persian Gulf, and the Caspian Sea (JONES and MANN 1963, HANELT 1992, FRITCH and FRIESEN 2002). However, the wild progenitor(s) and origin of common onion are still not fully understood. Due to their morphological similarities, the wild species *Allium oschaninii* O. Fedtsch (*A. cepa* var. *silvestre* Regel) from Central Asia has been for a long time believed to represent the wild ancestor of the common onion (STEARN 1980, HANELT et al. 1992). This hypothesis was rejected because of different heterochromatic (Giemsa C-banding) patterns of their karyotypes (VOSA 1976). Additional experiments also rejected that hypothesis: severe crossing barriers appear between *A. cepa* and *A. oschaninii* (VAN RAAMSDONK et al. 1992); the chloroplast phylogeny of the section *Cepa* placed *A. oschaninii* outside of the clade containing *A. cepa* (HAVEY 1992). Several recent molecular analyses indicated that the wild species *A. vavilovii* M. Popov et Vved. could be a possible progenitor of *A. cepa*. RFLP analysis of nuclear DNA revealed identical pattern in *A. cepa* and *A. vavilovii* (BRADEEN and HAVEY 1995) and the sequences of three chloroplast loci also indicated a close genetic relationship between *A. cepa* and *A. vavilovii* (VAN RAAMSDONK et al. 2000). One of the main items of evidence suggesting that *A. vavilovii* is a progenitor of the common onion was the phylogenetic study of internal transcribed (ITS) region of the nuclear ribosomal DNA (GURUSHIDZE et al. 2007).

By application of selection and crossings a number of different *A. cepa* forms and varieties have been produced. They can be grown in different climates, on every continent, except in the extreme northern regions and humid tropical and subtropical regions. Red onion cultivars are classified into three groups (BRICKELL et al. 2009, JONES and MANN 1963, FRITCH and FRIESEN 2002): a) Common onion group: underground bulbs are large, flattened at the poles, usually single, inflorescence without bulbils, and reproduction by seeds. This group includes the largest number of commercially produced cultivars, b) Aggregatum group: underground bulbs elongated with numerous lateral bulbs, no bulbils in the inflorescence, either producing seed or else sterile, and reproduction almost exclusively vegetatively, c) Proliferum group: underground bulbs often poorly developed, inflorescence bears bulbils, do not produce seeds and reproduce vegetatively by bulbils in the inflorescence.

The term shallot is most commonly used for a specific variety of *A. cepa*, and was formerly even classified as a distinct species, *A. ascalonicum* L. Recent studies have shown that the term shallot in southern Croatia is additionally used for diploid and triploid viviparous onions *A. × proliferum* (Moench.) Schrad and *A. × cornutum* Clementi ex Vis. (PUIZINA 1992, 1997). This article provides an overview of the results of studies on the morpho-anatomical and genetic structure. A common morphological feature of all three taxa is the vegetative propagation using underground bulbs and a close relationship with the common onion.

Shallot, *Allium cepa* Aggregatum group

Shallots had previously been considered a separate species, *A. ascalonicum*, but today they are classified within the common onion under the currently accepted name *A. cepa* Aggregatum group (RABINOWITCH and KAMENETSKY 2002). Besides the best known name *A. ascalonicum*, there are several other synonyms in use: *A. cepa* var. *aggregatum*, *A. cepa* var. *ascalonicum*, *A. cepa* cv. 'Shallot' (JONES and MANN 1963, HANELT et al. 1992, RABINOWITCH and KAMENETSKY 2002).

Although these plants look morphologically very similar to *A. cepa*, there are some differences. Plants are perennials and generally have smaller flowers, inflorescences, bulbs and leaves than *A. cepa*. Compared with the leaves of *A. cepa*, leaves of shallot are thin, tender, often bent and particularly flat, almost concave on the inner side of the leaves. Underground bulbs are well developed; their shape is oblong, semi-cylindrical. A large number of bulbs are gathered in the clusters. After planting each bulb develops an entire group of side bulbs that are interconnected. Each new bulb develops its own leaves, so that the whole plant has a bushy appearance. In contrast, *A. cepa* usually has a well-developed stem. The shallot cultivars examined by PUZINA (1992) had no stamen morphology typical of *A. cepa* (outer anthers simple, interior anthers with an expanded base and one small tooth on each side), but all six stamens were simple (Tab. 1).

All taxa of *A. cepa* Aggregatum group are diploids ($2n = 2x = 16$) (HANNELT et al. 1992). The structure of the shallot's karyotype could not be distinguished from the karyotype of *A. cepa*. The constitutive heterochromatin as identified by Giemsa C-banding is located mainly at the end of the chromosomes. Only a few interstitial C-bands were identified and their distribution was similar to the distribution of Giemsa C-banding in *A. cepa* (Figs. 4a, b) (VOSA 1976, PUZINA 1992). In meiosis eight bivalents are regularly seen in diakinesis and metaphase I (Fig. 5d). Bivalents have terminal type of chiasmata and are generally of the same shape as *A. cepa* bivalents (rings and rods) (Fig. 5a). The plant rarely produces flowers although the pollen is fertile and a seed is produced. It is mainly propagated vegetatively by dividing and planting underground bulbs.

On a global scale, the shallot, *A. cepa* Aggregatum group, is a minor *Allium* crop. However in South East Asia and some African countries where onion seed is hard to produce, where onion culture is difficult and where the growing season is too short for the production of bulb onion, the vegetatively propagated shallot is cultivated as an important substitute for bulb onion (RABINOWITCH and KAMENETSKY 2002). The relationship of shallots to common onion was investigated by application of Random Amplified Polymorphic DNA (RAPD) markers and morphological traits (LE THIERRY D'ENNEQUIN et al. 1997). Sexually propagated shallots were more closely related to common onion than the vegetatively propagated shallots, which grouped separately. The grey shallot is a specific form of shallots long cultivated in France and Italy. MAASS (1996) compared the isozyme profile of 30 accessions of the French grey shallot, with those of 466 bulb onions and other shallots, 15 accessions of *A. oschaninii* and 22 accession of *A. vavilovii*. The results suggested that the French grey shallots differ from other shallots and were more closely related to either to *A. oschaninii* or *A. vavilovii*, that to *A. cepa* and other shallots. According to the RAPD analysis and GISH results, FRIESEN and KLAAS (1998) also concluded that grey shallots belong to *A. oschaninii*.

Shallot, *Allium × proliferum* (Moench) Schrad.

In the local dialect of South Croatia, there are several different names for this shallot: kozjak, orijaš, škalonja, ljutika, ljutika-talijanka (PUZINA and PAPEŠ 1999). The diploid viviparous onion is also known as top onion, tree onion or Egyptian onion. Previously it has been classified as a variety of common onion *A. cepa* var. *proliferum* (Targioni, Tozzetti) or *A. cepa* var. *viviparum* (Metzg.) Alef. (HELM 1956, JONES and MANN 1963, McCOLLUM 1974, PUZINA and PAPEŠ 1996). Since plants represent a spontaneous hybrid between *A. cepa* (common onion) and *A. fistulosum* (Japanese bunching onion), SCHUBERT et al. (1983)

Tab. 1. Morphological characteristics of shallots in Croatia.

Morphological character	Shallots		
	<i>A. cepa</i>	<i>A. × cornutum</i>	<i>A. × proliferum</i>
Bulbs	<i>A. cepa</i> round, flattened at the poles, 5–10 cm in diameter	<i>A. × cornutum</i> semicylindrical, well developed and gathered in clusters, 2–5 cm diameter	<i>A. × proliferum</i> elongated, less developed, 2–4 cm in diameter
Stem	up to 100 cm tall, hollow, conical, inflated at the base, 3 cm diameter	up to 100 cm tall, hollow, conical, slightly inflated at the base, diameter 3	80–150 cm tall, hollow, conical, inflated at the base, 3–5 cm diameter
Leaves	up to 40 cm long, diameter up to 2 cm, semicircular and flattened on the upper side	up to 40 cm long, diameter up to 2 cm, tubular, circular in cross-section	up to 50 cm long, diameter up to 3 cm, semicircular and flattened on the upper side
Spathe	shorter than inflorescence, divided into 2–4 parts, mostly triflorous	shorter than inflorescence, divided into 2–4 parts usually triflorous	significantly longer than the inflorescence, 1–2 parts
Inflorescences	hemispherical, diameter, 4–9 cm	spherical to semispherical, with bulbils, diameter 4–5 cm	hemispherical, numerous bulbils and rare flowers, diameter 3–5 cm
Perianth	star like shape of a flower, segments white with a green stripe, size of segments 3–4.5 × 2–2.5 mm	star like shape of a flower, segments white with a green stripe, size of segments 3.5–4 × 3 mm	closed shape of a flower, campanulate, segments yellowish-white, size of segments 5–7 × 2 mm
Flower pedicels	equal, up to 4 cm long	equal 1–2 cm long	unequal, 0.3–2 cm long
Stamens	filaments 4.5 mm, outer simple, interior with extended toothed base.	filaments 3–4 mm, outer and inner simple	filaments 4–7 mm, outer and inner simple
Pistils	lower than the stamens	taller than the stamens	taller than the stamens

pointed out that for nomenclatural reasons this onion should be named *A. × proliferum* (Moench) Schrad.

Plants have a relatively poorly developed bulb, similar to the parental species *A. fistulosum*. A sort of gigantism that characterizes this cultivar is its most striking feature, in which it also differs from other varieties of onions. Due to the rapid vegetative development, these perennial plants can grow up in height up to 130 cm, and their flower pedicels can be 3–5 cm long. Their inflorescences regularly contain flower bulbils with some sterile flowers. Bulbils often sprout within the transformed umbel (Figs. 1c, f), sometimes in two or more tiers. With respect to the shape and structure of the stamen's filaments, the plant more closely resembles *A. fistulosum*. Specifically, the shape of the flower is more campanulate and closed as in *A. fistulosum*, rather than stellar and open as in *A. cepa*. Stamen filaments are long, thin, without or with barely noticeable expansion on the basis of internal stamens. Anthers are often rudimentary or sterile (Fig. 2d). Given the form of leaf, this type of shallot resembles *A. cepa* (flattened on the inner side). A special feature of this shallot is the long spathe that envelops the inflorescence, which it surmounts considerably. This novel feature is not characteristic of *A. cepa* or *A. fistulosum*. As the underground bulbs are poorly developed, and the plant does not produce seeds, plants mostly reproduce vegetatively by bulbils from the inflorescences (usually 4–8 bulbils per inflorescence).

Fig. 1. *Allium × cornutum*: **a** – appearance; **b** – plants in the soil; **d** – young inflorescence, **e** – mature inflorescence carrying two bulbils which are already sprouting. *Allium × proliferum*: **c** – plants in the soil; **f** – bulbils often sprout within the transformed inflorescence before its opening.

Top onions, *A. × proliferum*, are a vegetatively propagated garden crop in Europe, North East Asia and North America. Several karyological studies indicated its hybrid (allodiploid) nature and showed that the karyotype consists of one haploid *A. cepa* and one haploid *A. fistulosum* set of chromosomes (BOZZINI 1964, MCCOLLUM 1974, FISKESJO 1975, SCHUBERT et al. 1983, PUIZINA and PAPEŠ 1999). Chromosomes preserved their original C-banding pattern as in the parental species with the exception of *A. cepa* satellite chromosome that lost its satellite (Fig. 4d). Meiosis of *A. × proliferum* is characterized by chromosome pairing of nonhomologous chromosomes into heterobivalents (Fig. 5d). However, univalents were frequently seen and very rarely some multivalents. Bivalents with terminally located chiasmata dominate, but occasionally bivalents with localized chiasmata (near the centromere) can be seen (PUIZINA 1997). The hybrid origin has been proved by isozyme analysis (MAASS 1997a), RAPD markers (FRIESEN and KLAAS 1998) as well as by genomic *in situ* hybridization (GISH) experiments using labeled total genomic DNAs from *A. cepa* and *A. fistulosum* as probes. Each probe labeled one genome (eight chromosomes of top onion; FRIESEN and KLAAS 1998, PUIZINA and PAPEŠ 1999). *Allium wakegi* Araki is a sexually sterile ancient garden crop in Japan and China. Its hybrid nature (*A. cepa* × *A. fistulosum*) has been confirmed by GISH and by localization of 5S rRNA loci at chromosomal positions corresponding to *A. cepa* and *A. fistulosum* (HIZUME 1994).

Shallot, *Allium × cornutum* Clementi ex Vis.

The triploid shallot is traditionally cultivated in South and coastal Croatia under the name 'Ljutika' and it is very popular as a spice and condiment due to its tasty bulbs and leaves. It was first named *A. cepa* var. *viviparum* (LANGER and KOUL 1983; PUIZINA and PAPEŠ 1996, 1997), but FRIESEN and KLAAS (1998) suggested that this name is connected with the other viviparous onion *A. × proliferum* and proposed using the name *A. cornutum*

Fig. 2. Flower and stamen morphology: **a, b** – *A. fistulosum*; **c, d** – *A. × proliferum*; **e, f** – *A. × cornutum*; **g, h** – *A. cepa*. In contrast with *A. cepa*, which is characterized by the broadened base of the inner filament (**h**), the triploid *A. × cornutum* possesses rather pronounced triangular teeth at the bases of the inner filaments (**f**).

Clementi ex Vis. (VISIANI 1842), the only name which is unambiguously connected with the triploid onion (STEARN 1980, MAASS 1997b). However, taking into account its hybridogenic origin, it was modified to: *A. × cornutum*. Interestingly, this name was first used by VISIANI (1842) for a Dalmatian bulbiferous taxon, which was observed for the first time on the rocks of Dubrovnik (STEARN 1980).

In contrast to most flowering species of *Allium* in which the leaves are already dying back at flowering time, triploid shallots are perennials, their leaves remain green and suitable for use during entire year. It blooms, like the majority of other *Allium* species, from May and June. Plants are sterile and do not produce seeds. They propagate vegetatively by underground bulbs and bulbils from inflorescence. Phenotypically, triploid shallots (viviparous onions) closely resemble *A. cepa*, and sometimes it is difficult to distinguish them if they are planted both in the soil, before developing their inflorescences (Figs. 1 a, b). The triploid shallot's flower is star-shaped; the segments are white with a green stripe. Three inner stamens have a broadened base with two prominent teeth (one from each side) (Figs. 2e, f). Outer stamens are simple. Inflorescences of the triploid shallot initially contain only the flowers, which are by their dimensions slightly larger than the flowers of *A. cepa* and generally less numerous in the inflorescence than in *A. cepa* (Figs. 1a, 3a). During the maturation of inflorescences, small reproductive bulbils begin to appear (Figs. 1d, e) and flowers gradually disappear. At the end, the mature inflorescence is composed only of the bulbils (up to 20–30 bulbils per inflorescence). It has never been noticed that bulbils from the inflorescence develop their own stems and inflorescences, which is a regular case in the diploid hybrid shallot, while occasionally triploid bulbils from the inflorescences develop only a few leaves (Fig. 1e).

These small reproductive bulbils, which regularly develop among sterile flowers in the inflorescence of the triploid shallot are a reliable sign for its recognition, as they never develop in the diploid *A. cepa*. Another reliable sign of the triploid shallot as against *A. cepa* are the elongated underground bulbs (Fig. 1a). Bulbs are gathered in large numbers, sometimes 10–20 or more individual bulbs are held together by the same basal plate. From each bulb leaves emerge and the plant has a bushy form. The triploid shallot can also be distinguished from *A. cepa* with respect to the shape of leaves. Leaves are not semicircular and flattened on one side as they are in *A. cepa*, nor are they round in shape. They are intermediate between the semicircular and the round shape. The stalk that bears the inflorescence is only slightly flattened at the bottom, whereas the stalk in *A. cepa* is inflated at the base of the stem.

The triploid shallot does not exhibit the same effect of gigantism as top onions. It is even slightly smaller than the diploid *A. cepa* (they grow up to 100 cm). Although its bulbs and leaves are very tasty and are used in the diet, there is a problem of their prolonged storage and keeping after harvesting, because they tend to rather quickly produce new leaves and roots, thus losing weight. That is probably the reason why the bulbs are usually kept by being preserved in vinegar.

The karyotype of *A. × cornutum* ($2n = 3x = 24$) consists of 24 chromosomes (SINGH et al. 1967, PUZINA and PAPEŠ 1996). The homology between the chromosomes is weak and occasional, and it is very difficult, and even impossible, to identify homologous pairs of chromosomes. Therefore the chromosomes are usually lined up with respect to size. The exceptions are the three subtelocentric chromosomes, which are put at the end of the karyotype (Figs. 3a, b). The most common chromosome associations in diakinesis and metaphase I of

Fig. 3. Mitotic chromosomes of root tip cells in *A. x cornutum* (a), $2n = 3x = 24$, stained by Feulgen. Arrows indicate the three satellite chromosomes. Chromosomes aligned in the kariogram (b). Scale bar=10 μ m.

A. x cornutum are heterotrivaleents, which suggests a partial homology of the three genomes (PUIZINA and PAPEŠ 1997). Additionally, the frequent occurrence of complex multivalents was observed (4–11 paired chromosomes) suggesting that intergenomic pairing, translocations and chromosomal rearrangements were involved in the evolution of the triploid karyo-

Fig. 4. Schematic representation of Giemsa C-banding karyotypes in shallots.

type (Fig. 5e). Identification of the constitutive heterochromatin by Giemsa and fluorochrome banding in the karyotype of *A. × cornutum* (LEPEN and PUZINA 2011) indicated the hybrid structure of the karyotype, which contained only one genome originating from *A. cepa* (Fig. 4e). Previously, triploid viviparous onions have been suspected to be either an allotriploid (AAB) (SINGH et al. 1967), or a segmental allotriploid (AA'A'') (KOUL and GOHIL 1971). The chloroplasts DNA (cpDNA), the nuclear fragment of rRNA genes and the RAPD molecular markers indicated *A. cepa* as one parent (HAVEY 1991, 1992, 1993; KLAAS and FRIESEN 2002).

In order to determine the parental *Allium* species of *A. × cornutum*, genomic fluorescent in situ hybridization (GISH) was applied (PUZINA et al. 1999). Biotinylated genomic DNAs from six diploid *Allium* species (*A. cepa*, *A. fistulosum*, *A. roylei* Stearn, *A. vavilovii*, *A. galanthum* Kar. et Kir. and *A. oschaninii*) were used as probes. While probes obtained from genomic DNA of *A. cepa*, *A. vavilovii* and *A. roylei* hybridized to somatic chromosomes of *A. × cornutum*, probes from *A. fistulosum*, *A. galanthum*, and *A. oschaninii* did not. The DNA probes of *A. cepa* and *A. roylei* each completely or predominantly labeled one genome (eight chromosomes). A few chromosomes, the markers of the triploid karyotype, were not completely labeled by any of the probes applied. These GISH results indicated that triploid viviparous onions might possess a complex triparental genome organization (PUZINA et al. 1999). Using a similar approach FRIESEN and KLAAS (1998) reached a different conclusion. They could verify only *A. cepa* as a parental species and according to these authors, the majority of DNA and chromosomes of *A. × cornutum* originate from *A. cepa*.

Further progress in the identification of the parental species of *A. × cornutum*, an 'enigmatic plant' as noted by KLAAS and FRIESEN (2002), was hampered by the lack of more molecular data about wild relatives of common onion as well as suitable molecular markers. Recently, the ITS sequences (internal transcribed spacer of the 18S-5.8S-26S rDNA) of large number of common onion relatives originating from Central Asia, were deposited in GenBank (GURUSHIDZE et al. 2007) as well as the sequences of the 5S rRNA (SON et al.

Fig. 5. Meiosis, metaphase I: **a** – *Allium cepa*; **b** – *A. fistulosum*; **c** – *A. cepa* Aggregatum group; **d** – *A. × proliferum*; **e** – *A. × cornutum*.

2012). Our preliminary results detected several types of ITS and 5S rRNA sequences in *A. × cornutum*. Detailed analysis of these sequences is in progress and the results achieved will help us to understand the origin and the genome structure of that interesting triploid hybrid better.

The triploid viviparous shallot *A. × cornutum* is a rather widespread garden crop in South-East Asia, Europe and other parts of the world. Based on several morphological, cytogenetic and molecular studies, it is now recognized as a separate *Allium* crop and a new taxonomic unit. Since almost all its clones are practically identical (MAASS 1997b, FRIESEN and KLAAS 1998, PUIZINA et. al 1999), the monophyletic origin of this widely distributed crop is likely, and northern India (Kashmir) might be the place of origin. Although humans must have contributed to the maintenance and spread of this vegetatively reproduced crop, findings of *A. × cornutum* in abandoned vineyards and other less-favored agricultural and even landfill areas suggest that this plant is very tolerant to drought and poor soil and is able to persist even in wild habitats.

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