# Chapter 4
# Spring Onion (*Allium fistulosum* L.) Breeding Strategies


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**Abstract** Spring onion (*Allium fistulosum* L.) belongs to subgenus *Cepa,* genus *Allium* and family Liliaceae and popularly known as scallion, Welsh onion and Japanese bunching onion. Cultivation of spring onion dates back to 200 BC in China and reached Japan before 500 AD which later spread to Southeast Asia. Spring onion is grown worldwide, however the main area of cultivation remains in East Asia from Siberia to tropical Asia including China, Taiwan, Japan, the Philippines, Malaysia and Indonesia. The plant is a perennial herb, does not produce bulbs, and possesses hollow leaves and has traditionally been used in Chinese folk medicine to treat common cold, influenza, abdominal pain, headache and cardiovascular disease as well as having antifungal and antibacterial effects. Spring onion is known for its flavor and aroma and is a rich source of vitamin C, A and $B_6$, thiamine, folate, rhamnose, galactose, glucose, arabinose and xylose. Production of $F_1$ hybrids is considered as one of the main goals in crop breeding. The length of time taken is the main restriction in breeding programs as eight or more generations of inbreeding are needed to establish homozygous lines that can be applied in hybrid production. This process can be enhanced by using doubled haploid (DH) lines as components of hybrid cultivars. In this chapter, we give an overview of the origin, botanical classification, distribution, reported health benefits, genetic resource and conservation, crop cultivation practices and recent advances on biotechnology, and molecular biology and their application for crop improvement in connection with traditional breeding methods of spring onion. In this aspect, mutational breeding and somatic hybridization are the potential approaches for the development of new high-yielding and disease-resistant cultivars.

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## 4.1 Introduction

Spring onion (*Allium fistulosum* L.) belongs to the Liliaceae family and in some countries is known as scallion, green onion, Welsh onion, salad onion, Japanese bunching onion, Spanish onion, two-bladed onion and green trail (Fig. 4.1). Other local names include *cong* in China; *ciboule*, *oignon de Strasburg* in France; *rohtenlauch*, winter *zwiebel* in Germany; *negi* in Japan; *pa* in Korea; *pijplook*, *bieslook* and *indischeprei* in The Netherlands, *cebolla*, *ceboletta* in Spain and *chung* in Taiwan (Inden and Asahira 1990; Sang et al. 2002). *Allium fistulosum* is a perennial, herbaceous plant which is usually grown for its edible tops, long leaf bases or young shoots. It does not develop bulbs, and possesses hollow leaves (*fistulosum* means *hollow*) which differ from leek, where their leaves are flat. A large number of *A. fistulosum* varieties resemble the leek, such as the Japanese *negi*, while smaller varieties resemble chives. Leaves of *A. fistulosum* are somewhat rounder in cross-section, not flattened adaxially.

**Fig. 4.1** Spring onion sold at West End Market, Brisbane, Australia

### 4.1.1 Botanical Classification and Distribution

The inflorescence of spring onion lacks bracteoles and the size of the flower is about twice of *Allium cepa*. The scape is round, hollow, 40–75 cm tall, and does not have the typical bulge of onion scapes. Diameter of the umbel ranges from 3 to 7 cm and is not spherical. The order of opening of the pale yellow flowers begins at the top of the umbel and proceeds toward the base and becomes a distinguishing feature in contrast to most other alliums except chives (Rubatzky and Yamaguchi 1997). The filaments of the stamens are more protruded, they lack basal teeth and are not broadened at the base. This type of onion is harvested at an immature stage before the bulb has fully developed. Some useful characteristics in identifying different *Allium* vegetables are shown in Table 4.1. Their flavor and aroma much milder than red onion; they are eaten raw in salads and sandwiches. The green tops are used as a garnish or sliced in salads or stir-fries. It has been a common food to humans since the earliest times, along with garlic, leek, chive, bulb onion and shallot.

Spring onion belongs to subgenus *Cepa*, genus *Allium* and family Liliaceae; the basic chromosome number of *A. fistulosum* is 8; (2n = 2x = 16). The size of spring onion, *A. fistulosum* genome is estimated to be 11.7 pg/1C or $1.2 \times 10^4$ Mbp (Ricroch et al. 2005). It is self-compatible with a high degree of cross-pollination. Self-pollination may occur in some plants and show inbreeding depression and poor growth. Landraces and open pollinated varieties exhibit high levels of heterozygosity and heterogeneity (Tsukazaki et al. 2006; Wako 2016).

**Table 4.1** Useful characteristic in identifying *Allium* vegetables

| *Allium* | Chromosome number | Flower color | Bulb formation | Bulbils in inflorescence |
|---|---|---|---|---|
| Onion and shallot | 16 | White, green striped | Yes | Absent in most cultivars |
| Garlic | 16 | Lavender to pale green and white | Yes | Very common |
| Great headed garlic | 48 | White to purple | Yes | Usually not |
| Spring onion | 16 | Pale yellow to white | No | Absent in most cultivars |
| Chive | 16, 24, 32 | Purple or rose, rarely white | No | Rarely |
| Chinese chives | 32 | White | No | No |
| Rakkyo | 16, 24, 32 | Rose-purple | Yes | No |
| Leek and kurrat | 32 | White to purple | No | Sometimes |

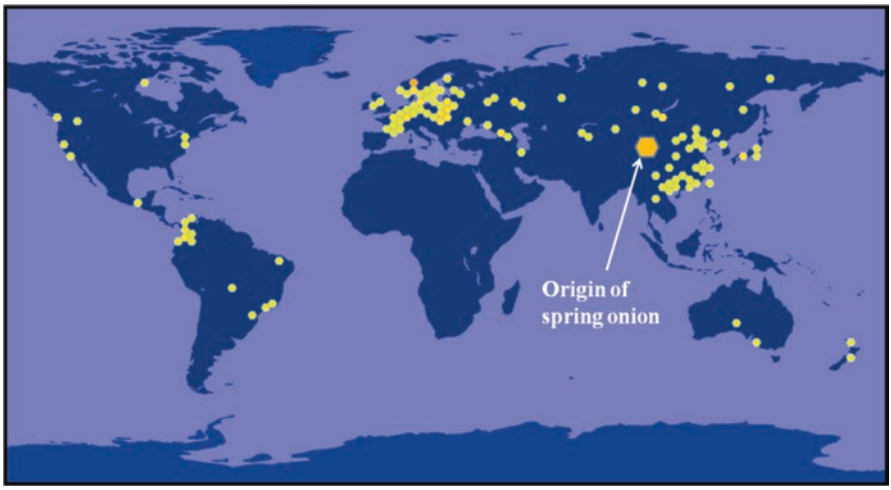


**Fig. 4.2** Map of origin and distribution of spring onion. Yellow dots indicate production sites

### *4.1.2 Origin and Domestication*

Traces of alliums have been found in the remains of Bronze Age settlements. Seven major edible *Allium* species from 500 to 650 varieties of *Allium* worldwide are among of the earliest domesticated crops for its flavor, medicinal and nutritional properties (Shigyo et al. 2018). Plants with the largest and quickest-growing bulbs were selected by early farmers which probably originated in northwestern China (Sang et al. 2002; Tsukazaki et al. 2010). Cultivation of spring onion dates back to 200 BC in China and reached Japan before 500 AD and later spread to Southeast Asia (Fig. 4.2). The earliest description of the crop and its cultivation is found in a Chinese book of 100 BC and mentioned the first time in Japanese literature in 720 AD (Inden and Asahira 1990).

## 4.2 Health and Economic Importance

### *4.2.1 Health Benefits*

*Allium fistulosum* has traditionally been used in Chinese medicine to treat the common cold, influenza, abdominal pain, headache and cardiovascular disease. According to a dictionary of Chinese drugs, the bulbs and roots are used for treatment of febrile disease, headache, abdominal pain (Yamazaki et al. 2016), diarrhea, snakebite, ocular disorders, habitual abortion, as well as having antifungal and antibacterial effects (Sang et al. 2002; Ueda et al. 2013). Extracts from spring onion are a potential source of natural antioxidants (Aoyama and Yamamoto 2007; Wang

et al. 2006) and antimicrobial agents (Chang et al. 2013). It is also reputed to improve eyesight, help in digestion, perspiration, recovery from wounds and festering sores. It was traditionally served to sick people as a preservative against *evil spirits*. Kang et al. (2010) suggested that their fibrous roots have potential for as a hypoglycemic agent in controlling blood glucose.

Several studies indicate that *Allium fistulosum* is a rich source of vitamin C, A and $B_6$, thiamine, folate, potassium, copper, manganese, iron and chromium (Table 4.2). Leaf blades are rich in rhamnose, galactose, glucose, arabinose and xylose (Inden and Asahira 1990). The organoleptic quality of leaf-bunching onions improves under low temperature, which increases well-hydrated gels of cellulose, hemicellulose, protopection and water-soluble pectin, which embed free sugars

**Table 4.2** Nutritional value of spring onion, *Allium fistulosum* per 100 g

| Spring onion (raw), Nutritional value (per 100 g) | | Daily values (%) |
|---|---|---|
| PROXIMATES | | |
| Energy (kJ/Cal) | 32 kJ (135 kcal) | 2 |
| Protein (g) | 1.83 | 4 |
| Fat (g) | 0.19 | n.a |
| Water (g) | 89.83 | n.a |
| Carbohydrate (g) | 7.34 | 2 |
| Sugars (g) | 2.33 | n.a |
| Dietary Fiber (g) | 2.4 | 10 |
| Ash (g) | 0.81 | n.a |
| VITAMINS | | |
| Folate, Vit. B9 (µg) | 64 | 16 |
| Thiamine, Vit. B1(mg) | 0.55 | 4 |
| Riboflavin, Vit. B2 (mg) | 0.08 | 5 |
| Niacin, Vit. B3 (mg) | 0.525 | 3 |
| Vitamin B6 (mg) | 0.061 | 3 |
| Vitamin A (IU) | 997 | 20 |
| Vitamin C (mg) | 18.8 | 31 |
| Vitamin E (µg) | 0.55 | 3 |
| Vitamin K (µg) | 207 | 259 |
| MINERALS | | |
| Calcium, Ca (mg) | 72 | 7 |
| Iron (mg) | 1.48 | 8 |
| Magnesium, Mg (mg) | 20 | 5 |
| Phosphorus, P (mg) | 37 | 7 |
| Potassium, K (mg) | 276 | 8 |
| Zinc, Zn (mg) | 0.39 | 3 |
| Sodium, Na (mg) | 16 | 1 |
| Copper, Cu (mg) | 0.083 | 4 |
| Manganese, Mn (mg) | 0.16 | 8 |
| Selenium, Se (µg) | 0.6 | 1 |

Source: USDA National Nutrient Database for Standard Reference (2016)

carbohydrates storage in the leaf (Hang et al. 2004; Inden and Asahira 1990). Flavor is attributed to the enzyme allinase which acts on sulfur compounds when the tissues are disrupted. The strength of flavor increases with plant age (Peffley 1992). Spring onion is known to contain amino acids and peptides such as cysteine, and glutathione which act as a redox agent in dough, improving bread making properties (Seguchi and Abe 2003)

Flavonoids, carotenoids and some sulfur compounds are phytonutrients found in spring onion. They are packed with highly effective flavonoids known for their health-promoting effects. Aoyama and Yamamoto (2007) studied the effect of thermal treatment on antioxidant activity and flavonoid content, and observed an increase in antioxidant activity and decrease in flavonoids of green spring onion compared to three other vegetables (yellow and red onion varieties and white-sheath spring onion) during the boiling procedure. The study suggested that green Welsh onion, but not the white one, is a potent antioxidant food comparable to yellow onion, and is a good source of kaempferol. It possesses anti-platelet, antioxidative, anti-hypertensive and anti-hyperlipidemic properties (Sung et al. 2015), and may lower cholesterol level and decrease the risk of heart attack and stroke. Several studies indicate that spring onion has excellent antibacterial and antifungal properties. Sung et al. (2018) suggested that *Allium fistulosum* extracts could be used as functional food materials for weight control in obesity. The roots contain allicin and diallyl disulfide and have the potential to reduce the body fat mass.

### *4.2.2 Economic Importance*

Spring onion is grown throughout the world, but the main area of cultivation remains in East Asia from Siberia to tropical Asia including China, Taiwan, Japan, the Philippines, Malaysia and Indonesia (Inden and Asahira 1990). It is an important ingredient in Asian cuisine particularly in China, Japan, Korea and Southeast Asia. In Japan, it is the second most important ingredient after *Allium cepa* but it stands first in China, where it is used in different dishes. Shredded green onions often make up the *green* part of the five colors in a traditional Korean and Japanese meal. It is used as flavoring marinade in *bulgogi* (Korean grilled beef) and to flavor kimchi. Negi, which is also known as Welsh or spring onion, is among the most important ingredient in Japanese cuisine. It is usually served with soba noodles and tofu and always found in miso soup, negimaki and widely used as a garnish in noodles or stir-fried. In Southeast Asia it is mainly grown for salads, or as an herb to flavor soups and other dishes. Dehydrated spring onion is used as an additive to pre-processed food such as instant noodles, potato chips and others. The young inflorescence is sometimes deep-fried and consumed as a snack. Fried *Allium* oil is widely used in traditional Chinese cooking and has recently grown in popularity in the food manufacturing industry (Zhang et al. 2019).

Statistical analysis spring onion production is often combined with other *Allium* spp. *Allium* vegetables gross production in 2014 were valued at USD 61,348 million

with 4% of it is contributed by green onions and shallots (Galmarini 2018). The main world production of this vegetable is in Japan, South Korea, China, and Taiwan ranking in their top ten vegetable crops. Bunching onion has the highest annual output of *Allium* crops in Japan in 2014 (Tsukazaki et al. 2017). The annual production in Japan is about 500,000 mt from 23,000 ha, whereas in South Korea, around 27,000 ha produces 723,000 mt, and in China, 20,754,000 mt from 545,000 ha. The main production areas are distributed in the southern parts of these countries (Galmarini 2018). Data on annual consumption per person shows their consumption is 6.6 kg/capita/year in Korea, 1.7 kg/capita/year in Japan and 5.1 kg/capita/year in China (Galmarini 2018). Meanwhile, the annual production in Indonesia in 1988 amounted to 163,000 mt from 24,500 ha. In the USA, they are grown mainly in Monterey, Riverside, and Ventura counties in California and in Arizona, Georgia, Idaho, New Mexico, Oregon, Texas and Washington. In South America, Colombia has a considerable production of Japanese bunching onion for domestic consumption (Galmarini 2018). It is the second largest vegetable crop produced in Colombia, with 327,000 mt from 17,000 ha.

Given the enormous area of cultivation, great adaptability to climate and varied uses, a very large number of cultivars exist. Each cultivar has a characteristic commercial value due to the proportion of green leaves and white sheathes (Yamazaki et al. 2016). The Kumazawa system, based on utilization and ecological characteristic, is widely accepted as a method to classify spring onion in Japan. The system divides the Japanese bunching onion into four groups. *Kaga* which show little tillering and is dormant in winter, has dark green thick leaves and is grown for the pseudostems. *Kujyo* which remains green during winter; its cold tolerance is generally low and it has tender, green leaves of excellent eating quality; they tiller profusely and are mostly grown for their green tops. *Senju* which continues to grow during winter, although at a reduced rate, is intermediate between the former two and is mainly grown for the pseudostem. Meanwhile, *Yaguranegi* produces numerous tillers in spring and summer but its growth stops in winter, produces no flowers but only bulbils; it is propagated by division of the basal cluster or bulbils (Inden and Asahira 1990).

Spring onion is among the 15 most important crops in China. It is widely cultivated in Shandong, Henan, Hebei and Shaanxi provinces (Wang et al. 2018). It is preferred compared to bulb onion as it has a stronger flavor to enhance Chinese cuisine. There are more than 200 cultivars of Chinese spring onion which are classified as long pseudostem type, LP (long pseudostem, soft leaves, low soluble solids concentration and mild flavor), fleshy root types, FR (fleshy roots, high soluble solids concentration, stronger pungency and larger sheath diameter) and short pseudostem type, SP (intermediate between the other two types) (Liu et al. 2009).

On the island of Java, Indonesia, the three types of spring onion planted are *bawang bakung* which is grown for the pseudostem; *bawang cina* for the leaves and *bawang daun* which is the intermediate between the two. Among the popular Indonesian cvs. are Plumpung, Mambo, Nyonya, Siih Kecil and Tosari. Mixed cropping with white cabbage, carrot and potato is common in the highlands (Sulistriarini et al. 2016).

The main varieties of spring onion grown in Australia are Straight Leaf, Dynasty Winter King and Summer King. The immature bulb of bulb onion (*Allium cepa*) varieties such as early Lockyer White, South Australian White, Savages White and Gladalan White are sold as spring onion if harvested early with leaves intact. It is estimated that about 3 million bunches, or 1000 mt, of spring onion are produced from the Swan Coastal Plain, mainly between Wanneroo and Baldivis, in Western Australia (Burt 2007).

## 4.3 Current Cultivation Practices

Spring onion is easy to grow. It maintains vegetative growth all year round except in winter. Propagation is generally by seed and/or transplanted as seedlings in early spring, summer or autumn (Zhu et al. 2019). The crop produces tillers and can be propagated by seed or division. Division is preferred by most growers as it ensures that they have the chosen clone, especially where winter hardiness is an important requirement. Although it grows throughout the year, the best quality and highest yields are produced in late spring. Żurawik et al. (2013) showed that the highest marketable yield obtained from seeds sown at the end of April compare to early April in cv. Sprint. In temperate areas, propagation is mainly by seed, sown directly in the field or in nurseries before being transplanted into the field. Seed requirements are 8–16 kg/ha for direct seeding while 2–4 kg/ha for transplanting. In nursery beds, seeds are either broadcast or sown in rows or in 5–6 cm wide bands. Seedlings are ready for transplanting at about 15 cm height when it has pencil-thick base.

In Southeast Asia the crop is propagated mainly using basal tillers and can be planted the whole year round. Plants are rarely raised from seed as imported cultivars are more difficult to sow under tropical conditions and more time-consuming. In Indonesia, spring onion is planted in uplands as well as on dry paddy fields. In Java, it grows well above 200 m elevation, but it is more common above 500 m. Tillers are transplanted into raised beds or ridges, which are alternated with furrows for irrigation and drainage. There are many local selections and commercial cultivars, reflecting the adaptation to a wide range of climatic conditions. Most spring onion cultivars are well adapted to variations in rainfall and more tolerant of heavy rainfall than other *Allium* spp.

In Poland, spring onion is grown on a small scale (Majkowska et al. 2014) and mostly cultivated as a perennial crop out of rotation for the period of 3–4 years. Cultivars with a short pseudostem, strong tendency to tillering, abundant foliage production and fast regrowth after harvest like Siedmiolatka Czerwona, Kroll, Vita or Flamenco, are usually recommended beside other high yielding cultivars including Parade, Sprintesa, Performer, Ishikura Long White and Totem (Adamczewska-Sowinska and Kolota 2014). In Brazil, spring onion is grown throughout the year in highland regions and during autumn-winter in lower regions. Cultivars grown

include Todo Ano, Nebuka, Evergreen, Natsu Hosonegui, Futonegui and Ibirité (Marcuzzo and Carvalho 2014).

The main advantage of growing from transplants is the possibility of obtaining earlier yields (Tendaj and Mysiak 2011). Seedlings are planted in furrows, and roots and bases are lightly covered by soil. The depth of the furrow is about 15 cm for pseudostem production and 5 cm for green top production. Distance between rows and within rows are 55–85 cm and 3–15 cm, respectively, depending upon tillering tendency (Warade and Kadam 1998). Single plants are grown in a square spacing 20 × 20 cm or 25 × 25 cm depending on the vigor of the clone. Seedlings are planted in groups of 3 or 4 approximately 20 cm each way when using seed (George 2011). Highest yield was obtained in cv. Sprint at a sowing rate of 8 kg/ha$^{-1}$ and grown in rows 20 cm apart (Żurawik et al. 2013). Spring onion can be produced in most types of soil such as sandy loam, loam and clay loams with an optimum soil pH of 5.3–5.8. However, it is more successful at higher elevations (>1000 m) than lower. Ideally, it will grow efficiently in well-drained good fertile soil with good potential moisture retention as it has a sparse, shallow root system, and is easily rooted in waterlogged soil. Sprinklers are typically used, while drip irrigation is uncommon because of close row spacing. Typically, 25–38 cm of water is needed to meet evapotranspiration requirements (Smith et al. 2011).

Recommended rates of fertilizers in cultivation for branched pseudostems on soil with high organic matter is 200–300 kg of nitrogen, 100–200 kg of $P_2O_5$, and 150–200 kg of $K_2O$ per ha (Kolota et al. 2013). In greenhouse studies spring onion performed best when nitrogen was supplied as nitrate, $NO_3^-$. Complete fertilizer is usually used during planting time followed by two or three additional applications of nitrogen fertilizer. The use of high N rates to increase large onion bulbs and excessive nutrient supply is common in intensive farms to maximize marketable yield. Due to spring onion's poor root system, this practice may cause the risk of nitrate leaching. Therefore, the application of N fertilizer at the optimum recommended rate is essential in spring onion production (Liu et al. 2009).

A power tiller and a fertilizer applicator-ditcher was developed to reduce labor and cultivation costs, as the task can be completed so much faster than by the conventional way (Katahira et al. 2006). Growth, yield, flavor intensity and nutritional value of spring onion can be highly influenced by genotype, sulfur nutrition, soil type, climatic conditions, edaphic factors and management practices (Abbey et al. 2015). Sulfur fertilization reduces the total soluble solid content by 30% in spring onion cvs. Sydney Bunching as compared to Paris Silverskin. It also reduced the bulb diameters of *Allium fistulosum* cvs. Fragrant and Sydney Bunching, while increasing the bulb diameters in *A. cepa* cvs. Winter White Bunching, Egyptian Bunching, Winter Over and Paris Silverskin (Abbey et al. 2002).

Kołota et al. (2012) found that similar yields can be produced by spring onion cv. Performer harvested in early June to September. Meanwhile, lower yields with a gradual decrease of dry matter, carotenoids, sugars, volatile oils and nitrates have been produced from those harvested in October. Delay in harvesting at 60–120 days after planting results in a substantial yield increment with a simultaneous depletion of vitamin C, carotenoids, chlorophyll a and b, sugars, volatile oils, nitrates, total N,

K and Ca content. Dry matter content in spring onion cv. Sprint yield depends significantly on the sowing date with seed sown in May having higher dry content than those sown in April (Żurawik et al. 2013). Tendaj and Mysiak (2011) showed that spring onion grown from transplants produce longer and wider diameter pseudostems compared to seeds sown directly in the field.

*Allium fistulosum* usually depends on arbuscular mycorrhizal (AM) fungi for P uptake, as the adventitious root system is shallow, especially in soils with a low to medium P level. Tawaraya et al. (1996) suggested that selection of suitable fungal species and optimal phosphate application is important for spring onion growth. High dependency on mycorrhizal colonization has been observed in cvs. Sydney Bunching, Winter Over, Vilr-Moscow, Ciboule 9379 and Kagoshimahanegi (Tawaraya et al. 2001). Perner et al. (2007) suggested that AM colonization may support the production of organosulfur compounds by compensating growth depression caused by changes in N-form ratios in field conditions.

Suggested field planting density is 400,000–500,000 plants per ha, depending on the production of green leaf or pseudostem, respectively. Green leaves can be harvested 2–3 month after transplanting. However, it will take about 6–9 months to harvest for blanching (Rubatzky and Yamaguchi 1997). Plants are ready to harvest in 8–10 weeks in summer and 12–14 weeks in winter (Burt 2007). Spring onion competes poorly with weeds as they initially grow slowly. Hand weeding is required but it is costly. Harvesting can be coupled with replanting, separating plantlets at intervals to ensure continuity of supply. The production requires a lot of manual labor as each onion must be lifted by hand, trimmed, cleaned and packed into bundles. Several efforts have been made by a number of researchers to develop customized machinery to enhance farm production. Mechanized lifters and trimmers have been developed to speed the harvesting process.

Spring onion varies according to the cultivar in both juvenile age and cold requirement. Su et al. (2007) studied genotypic differences in the interaction between low temperature and photoperiod of spring onion cvs. Chunwei, Changbao and Zhanqiu, and found that each suffered from stress at low temperatures during winter, but differed in their response; Chunwei is the most tolerant, followed by Changbao, while Zhangqiu is the least cold tolerant. Greenhouse culture and plug seedling transplanting of spring onion are common in Japan. It is usually cultivated continuously with other crops without rotation in Hokkaido. Seedlings are raised in the greenhouse for up to 2 months before being transplanted into open fields from late April to mid-June and being harvested 4 months later (August to October). Leaf sheaths of the plants are covered with soil three times by hilling every month to promote etiolated growth. Some farmers cultivate the plants in greenhouses from autumn to the following spring (Misawa et al. 2017).

Vernalization is important in spring onion in order to prevent bolting as it may lower the yield while seed producers prefer to induce flowering to speed up the seed production. Flower induction is controlled by temperature and day length. Low temperatures (generally less than 13 °C) and short days induce flowering when a seedling has more than 11 leaves or is more than 5–7 mm in pseudostem diameter. Specific seedling characteristics and sufficient time at low temperature are required

for bolting. The temperature and time for vernalization may vary with cultivar. Plants are not vernalized when they are grown at more than 20 °C (Inden and Asahira 1990) and under a photoperiod of 16 h. With certain Taiwanese cvs. like Pei Chung, 5 days at 5 °C or 20 days at 10 °C are sufficient for vernalization. Dong et al. (2013) showed that cultivar, sowing date, transplant location and their interactions could influence bolting in spring onion. Bolting-susceptible cultivars are sown in late September to avoid vernalization temperature during winter while bolting-resistant cultivars can be sown earlier. They suggested that sowing around October and transplantation into the open field could delay bolting in cv. Xia Hei, while sowing in late August and transplantation to a plastic tunnel could accelerate flower bud development for seed production. Two mid-season flowering cvs., Kincho and Asagi-kujo, exhibited similar responses to temperature in flower initiation and bolting. However, after flowering was initiated, the early stage of flower development is day-neutral while a long-day photoperiod promotes flower development after the floret formation stage, followed by elongation of the seed-stalk (Yamasaki et al. 2011).

## 4.4 Pest and Disease Management

Although spring onion is generally a healthy crop, there are several bacterial and fungal diseases common to most *Allium* crops. Several problems regarding yield have occurred due to purple blotch (*Alternaria porri*), which causes concentric spots on the leaves, and downy mildew (*Peronospora destructor*). Most commercial growers follow guidelines for sanitation, crop rotation, use of resistant varieties and frequent monitoring to avoid severe disease outbreaks.

Successive cropping results in white rot (*Sclerotium cepivorum*) infestation as the pathogen is very persistent in the soil. Poor nutrition and heavy rains also can stimulate the disease development. In Victoria, Australia, vegetable growers report that this disease has progressively increased over the years due to frequent use of spring onion monocultures in short rotations with other non-host crops (radish, endive, parsley). Drastic increases of pathogen (sclerotia) populations in the soil lead to high disease levels and considerable yield losses which limit spring onion production. Rot commonly range from 5% to 50%, but in some seasons when soil conditions are favorable for disease development over 80% of plants may be killed (Villalta 2005).

Onion yellow-dwarf virus (OYSV), cycas necrotic stunt virus (CNSV) and tomato spotted wilt virus (TSWV) are pathogenic to Japanese bunching onion (Inden and Asahira 1990). The most important virus disease caused by the onion yellow dwarf virus is transmitted by various aphid species causing mosaic-type symptoms, including chlorotic mottling, streaking and stunting, and distorted flattening of the leaves. The Kujyo group of cultivars was found to be tolerant to this disease. The most serious pests are beet army worm (*Spodoptera exigua*) and the American bollworm (*Heliothis armigera*). They are difficult to control due to the

waxy layer on the leaves and that the larvae hide inside the hollow leaves (Sulistriarini et al. 2016).

Onion thrips (*Thrips tabaci* L.) is one of the most important pests in spring onion causing both direct and indirect damage by feeding and ovipositing on leaves that may cause green onions (scallions) to be unmarketable and dry bulb onion size to be reduced. Rust *(Puccinia allii)* causes serious damage to the leaves. Purple blotch or alternaria leaf spot (*Alternaria porri*) causes heavy losses under moist conditions. Phytophthora blight (*Phytophthora nicotianae* var. *parasitica)* leaf spot *(Pleospora herbarum),* black spotted leaf blight *(Septoria alliacea),* botrytis leaf spot (*Botrytis cinerea)* and fusarium wilt (*Fusarium oxysporum*) are fungal diseases which may affect the crop. Although a parental line with rust resistance has been successfully developed (Wako et al. 2012; Yamashita et al. 2005), it shows only a moderate level of field resistance to rust and does not completely control the disease. Li et al. (2018) reported that cultivation of cucumber with Welsh onion as a companion plant reduces root knots and egg masses of root-knot nematodes (*Meloidogyne* spp.) by 77%.

## 4.5 Germplasm Resources

Crop wild relatives (CWR) provide breeders with useful traits for crop improvement in a wide range of crops. Collection of germplasm of landraces and wild relatives creates a broad pool of potential genetic resources for breeding and innovative research (Hajjar and Hodgkin 2007). Improvement in gene bank information systems has allowed for better storage and management of a large quantity of data. The genus *Allium* is widely distributed over different regions from the dry subtropics to the boreal zone. Evolution of the genus coevolved with ecological diversification (Fritsch and Friesen 2002). Major efforts to collect source material of spring onion for evaluation and selection, and several trials have resulted in the economically-valuable Premyera variety of Welsh onion in southwestern Siberia (Shishkina et al. 2019).

Spring onion has resistance to several diseases that affect common onion as well, and it exhibits broad adaptability to a wide range of climatic conditions, winter-hardiness and early flowering. Cultivated germplasm forms the primary gene pool of the crop. This clearly indicates the potential for conserving genetic variation in landraces of the species (Ford-Lloyd and Armstrong 1993). Collections of *Allium fistulosum* exist in The Netherlands, Japan, UK, USA, Germany and the former USSR. A total of 975 accessions of *A. fistulosum* are conserved in botanical gardens and gene banks from 18,539 accessions of cultivated *Allium* genetic resources held worldwide (Table 4.3). About 89% of the *Allium* genetic resources are cultivated species (Keller and Kik 2018) with many economically-important species belonging to subgenus *Cepa*, e.g. *A. cepa* (onion, shallot), *A. fistulosum* (bunching onion) and *A. schoenoprasum* (chives).

**Table 4.3** Number of accessions of cultivated *Allium* species (sensu Fritsch and Friesen 2002) conserved per subgenus and in botanical gardens and gene banks

| Subgenus/species | Number of accessions | Botanical garden | Gene bank |
|---|---|---|---|
| ***Allium*** | | | |
| *ampeloprasum* L. | 2013 | 161 | 1852 |
| *sativum* L. | 4634 | 130 | 4504 |
| *macrostemon Bunge.* | 23 | 14 | 9 |
| *rotundum* L. | 127 | 30 | 97 |
| ***Cepa*** | | | |
| *fistulosum* L. | 975 | 105 | 870 |
| *altaicum* Pall. | 136 | 42 | 94 |
| *cepa* L. | 8660 | 215 | 8445 |
| *chinense* G. | 33 | 16 | 17 |
| *oschaninii* O. | 48 | 18 | 30 |
| *pskemense* B. | 66 | 38 | 28 |
| *schoenoprasum* L. | 607 | 256 | 351 |
| *proliferum* Schard. | 81 | 0 | 81 |
| ***Amerallium*** | | | |
| *canadense* L. | 49 | 38 | 11 |
| *hookeri* Thwaites. | 15 | 11 | 4 |
| *kunthii* G. | 12 | 7 | 5 |
| *neapolitanum* Cyr | 117 | 76 | 41 |
| *ursinum* L. | 177 | 103 | 74 |
| *wallichii* Kunth. | 30 | 26 | 4 |
| ***Anguinum*** | | | |
| *victorialis* L. | 150 | 104 | 46 |
| ***Butomissa*** | | | |
| *ramosum* L. | 103 | 45 | 58 |
| *tuberosum* Rottl. | 299 | 142 | 157 |
| ***Polyprason*** | | | |
| *obliquum* L. | 63 | 42 | 21 |
| ***Rhizirideum*** | | | |
| *nutans* L. | 121 | 60 | 61 |

Knowledge of genetic diversity aids in efficient management of germplasm and selection of parents for crossbreeding. Protein electrophoresis provides a source of marker genes for systematic studies within and between populations. Utilization of genes and their products such as isozymes and allozymes have been used to address issues dealing with local mating patterns, fine-scale structure within populations and broad-scale variation across species (Parker et al. 1998). Several studies using isozymes in *Allium fistulosum* are shown in Table 4.4. Most allozymes represent codominant Mendelian loci. However, their number is limited, and different taxonomic groups may exhibit variation and lack of polymorphism. It may also differ in metabolic function, and gene product could be exposed to selective processes in nature (Parker et al. 1998).

**Table 4.4** Isozymes used in *Allium fistulosum*

| Isozyme | Purpose | Country | References |
|---|---|---|---|
| *Pgm-1* and *Adh-1* | Mapping of enzyme coding genes *Adh-1 and Pgm-1* | USA | Peffley and Currah (1988) |
| *Got*, *Idh*, *Pgi* and *Pgm* | Intraspecific differentiation and isozyme pattern in *A. wakegi* | Japan | Okubo and Fujieda (1989) |
| *Adh-1* | Introgression of *A. fistulosum* L. into *A.cepa* L. | USA | Peffley and Magnum (1990) |
| *Idh-1*, *Adh-1* and *Pgi-1* | Study of isozymes in progeny of (*A. fistulosum* × *A. cepa)* × (*A. cepa*) | USA | Cryder et al. (1991) |
| *Got-1 and Got-2* | Study of isozymes in progeny of (*A. fistulosum* × *A. cepa)* × (*A. cepa*) | Netherlands | van der Valk et al. (1991) |
| *Acp-1*, *Acp-3, Est-3*, *Got-1, Got-2* and *Lap-1* | Study of isozyme genes in *A. fistulosum* | Japan | Haishima and Ikehashi (1992) |
| *Pgm-1, Adh-1, Acp-1, Acp-3, Lap-1, Got-1 and Got-2* | Study of isozymes in *A. fistulosum* | Japan | Haishima et al. (1993) |
| *Adh-1*, 6-*Pgdh-1*, 6-*Pgdh-2, Pgm-1* and *Skdh-1* | Study of isozymes inheritance in *A. fistulosum* | USA | Magnum and Peffley (1994) |
| *Got-1 and Got-2* | Mapping of glutamate oxaloacetate gene loci | Japan | Shigyo et al. (1994) |
| *Pgi-1* | Genetic analysis of *Pgi* isozymes in *Allium* subgenus *Cepa* | Japan | Shigyo et al. (1996) |
| *Lap-1, Got-1, Got-2 6-Pgdh-2, Idh-1, Pgi-1, Adh-1 and Gdh-1* | Identification of monosomic addition lines from *A. cepa* in *A. fistulosum* | Japan | Shigyo et al. (1996) |
| *Est-1* | Tacking the introgression between *A. fistulosum* and *A. cepa* | USA | Hou et al. (2001) |
| *Gdh-1* | Study of alien chromosomes transmission in self progenies of *A. fistulosum* × A. *cepa* | Japan | Shigyo et al. (2003) |
| *Got-2* | Characterization of alien chromosome addition in shallot from *A. fistulosum* | Japan | Hang et al. (2004) |
| *Lap-1, Got-1, Got-2, Pgi-1* and *Gdh-1* | Identification of alien chromosome addition in shallot from *A. fistulosum* | Japan | Yaguchi et al. (2009) |
| *AAT, CAR, Est, MDH, ME, SOD* | Isozyme variation in genus *Allium* | India, Bangladesh | Mukherjee et al. (2013) |
| *Lap-1, Got-1, Got-2* | Study of rust resistance using alien chromosome addition lines from *A. cepa* into *A. fistulosum* | Japan | Wako et al. (2015) |
| *Lap-1, 6-Pgdh-1, Pgi-1, Got-1 and Gdh-1* | Characterization of alien chromosome addition in *A. fistulosum* from *A. roylei* | Japan, Vietnam, Russia | Ariyanti et al. (2015) |

Notes: *Pgm* phosphoglucomutase, *Adh* alcohol dehydrogenase, *Pgi* phosphoglucoisomerase, *Acp* acid phosphatase, *Est* esterase, *Got* glutamate oxaloacetate transaminase, *Lap* leucine aminopeptidase, *Gdh* glutamate dehydrogenase, *6-Pgdh* 6-phosphogluconate dehydrogenase, *AAT* aspartate aminotransferase, *CAR* carbonic anhydrase, *MDH* malate dehydrogenase, *ME* malic enzyme, *SOD* superoxide, *Skdh* shikimate dehydrogenase, *Idh* isocitrate dehydrogenase

Phylogenetic relationships and evolution within *Allium* species have been investigated by several researchers. Molecular marker technology is been widely used for diversity analysis, varietal identification and facilitates selection of certain agronomic traits for crop improvement such as their polymorphic nature, natural behavior, easy and fast assay preparation, high reproducibility and easy exchange of data between laboratories (Chinnappareddy et al. 2013). Restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers are widely used for diversity analysis, varietal identification and study of DNA transfer within species in *Allium* (Table 4.5). Wilkie et al. (1993) reported the use of RAPD for diversity analysis of seven cultivars of onion and a single cultivar each of spring onion, chive, leek and *A. roylei* (a wild relative of onion). Application and impact of molecular markers on evolutionary and diversity studies in the genus *Allium* was reported by Klaas (1998) and Klaas and Friesen (2002).

Chloroplast and mitochondrial DNA are useful for the study of hybridization and introgression as they are inherited in a non-Mendelian, cytoplasmic fashion which usually relates to maternal transmission (Parker et al. 1998). Bark et al. (1994) demonstrated the use of RFLP in the chloroplast and nuclear genomes to assess DNA transfer from spring onion to bulb onion. Bradeen and Havey (1995) reported maternal phylogenetic relationship for *Allium altaicum, A. fistulosum, A. cepa* and *A. vavilovii* using RFLP of the chloroplast DNA. Use of mitochondrial DNA to distinguish cytoplasm of cultivated and wild *Allium* in subgenus *Cepa* was reported by Yamashita et al. (2000). RFLP analysis of mtDNA and cpDNA of two cultivated species of *A. fistulosum* and *A. cepa* along with four wild species in subgenus *Cepa* by Yamashita et al. (2001) demonstrated that phylogenetic relationships among the species closely correspond to the crossing ability test reported by Van Raamsdonk and Vries (1992). Yusupov et al. (2019) published a complete chloroplast of *A. fistulosum* consisting of 82,237 bp long single copy and 17,907 bp small single copy regions separated by 26,510 bp inverted repeat regions which could later be used for population genomic studies, phylogenetic analysis and genetic engineering studies of the genus *Allium*.

Nuclear DNA contains both unique single copy regions which generally code for a gene product and nonunique repetitive regions which consist of core sequences repeated in varying degree. Phylogenetic reconstruction based on nuclear DNA of *Allium* in subgenus *Cepa* was presented by Van Raamsdonk et al. (1997). A study using RAPD and RFLP by Friesen et al. (1999) showed that *A. fistulosum* originates from an *A. altaicum* progenitor. This result is contradictory to results published by Bradeen and Havey (1995) suggesting that phylogenetic hypotheses partly depend on the marker systems used. Development of RAPD marker for phylogenetic study in *Allium* subgenus *Cepa* has been reported by Shigyo et al. (2002). A study by Ricroch et al. (2005) revealed a significant interspecific variation in the amount of nuclear DNA and GC content of different onions and their wild allies. A phylogeny was constructed using internal transcribe spacer (ITS) sequences of 43 accessions representing 30 *Allium* species belonging to 3 major subgenera and 14 sections

**Table 4.5** Molecular markers used for diversity analysis and fingerprinting in *A. fistulosum*

| Marker type | Purpose | Country | References |
|---|---|---|---|
| Random-amplified polymorphic DNA (RAPD) | Genetic analysis in *Allium* | UK | Wilkie et al. (1993) |
| Restriction fragment length polymorphism (RFLP) | Analysis of *A. fistulosum* × *A. cepa* hybrid progeny | USA | Bark et al. (1994) |
| Restriction fragment length polymorphism (RFLP) | Phylogenetic relationship between *Allium* species | USA, Japan | Bradeen and Havey (1995) and Yamashita et al. (2001) |
| Random-amplified polymorphic DNA (RAPD) | DNA-based phylogenies in *Allium* subgenus *Cepa* | Netherlands | Van Raamsdonk et al. (1997) |
| Random-amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) | Evolutionary and diversity studies of *Allium* species | Germany | Klaas (1998) and Klaas and Friesen (2002) |
| Random-amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) | Phylogenetic analysis of *A. altaicum* and *A. fistulosum* | Germany | Friesen et al. (1999) |
| Restriction fragment length polymorphism (RFLP) | Study of mitochondrial DNA in *Allium* subgenus *Cepa* | Japan | Yamashita et al. (2000) and Yamashita et al. (2001) |
| Random-amplified polymorphic DNA (RAPD) | Development of RAPD in *Allium* subgenus *Cepa* | Japan | Shigyo et al. (2002) |
| Internal transcribe spacer (ITS) region | Phylogenetic and diversity studies of *Allium* species | Germany, France | Ricroch et al. (2005), Friesen et al. (2006), and Gurushidze et al. (2007) |
| Simple sequence repeat (SSR) | Intraspecific $F_1$ hybrid and interspecific taxonomic analysis in *Allium* subgenus *Cepa* | Japan | Araki et al. (2009) |
| Inter-simple sequence repeats (ISSR) | Species relationship study among *Allium* species | Korea | Son et al. (2012) |
| Amplified fragment length polymorphism (AFLP) | Development of a comparative genomic database for *Allium* | New Zealand | McCallum et al. (2012) |
| Simple sequence repeat (SSR) | Identification of cytogenetic marker for monitoring of alien genetic material | Russia, Belgium | Kirov et al. (2017) |
| Simple sequence repeat (SSR) | Study of the chloroplast genome of *A. fistulosum* | China, Uzbekistan | Yusupov et al. (2019) |

showed a tendency towards a decrease in genome size within the genus. Friesen et al. (2006) divide *Allium* into 15 subgenera and 56 sections based on internal transcribed spacer region (ITS) of nuclear ribosomal DNA with an estimation of 780 *Allium* species currently being recognized. Phylogenic analysis of *Allium* subgenus *Cepa* by Gurushidze et al. (2007) using sequences of the nuclear ribosomal DNA

internal transcribed spacer (ITS) region showed that the subgenus is monophyletic with three species group consisting (a) *A. altaicum*/*A. fistulosum*, (b) *A. farctum*/*A. roylei*/*A. asarense*/*A. cepa*/*A. vavilovii* and (c) *A. galanthum*/*A. oschaninii*/*A. praemixtum*/*A. pskemense.*

Tandem repeats are usually associated with chromosomal landmarks such as centromeres, telomeres, subtelomeric and other heterochromatic regions can be used for chromosome identification and to study plant chromosome evolution in many crops. Son et al. (2012) used ISSR analysis to study the relationships among *Allium* species. A comparative genomic database for genetic mapping and marker data from *Allium* species and population reported by McCallum et al. (2012) provide a valuable resource for genetic and genomic studies of *Allium* genome including *A. fistulosum*. Kirov et al. (2017) reported two cytogenetic markers, *HAT58* and *Cat36* for identification of individual chromosomes in *A. fistulosum*. Development of SSR markers for identifying intraspecific $F_1$ hybrids and interspecific taxonomic analysis in *Allium* subgenus *Cepa* are reported by Araki et al. (2009).

## 4.6 Breeding Strategies

### *4.6.1 Traditional Breeding*

Most cultivated alliums including spring onion are seed propagated (Havey 2002). Traditionally farmers raise their own seed or planting material. Breeders aim to improve cultivar homogeneity and adaptation to specific ecological conditions. High consumer and farmer preferences of low pungency, high sugar content, disease resistance, high yield, delayed bolting and suitability for mechanization are among the main important trait in spring onion breeding (Tsukazaki et al. 2017). Miyagi et al. (2011) showed that general consumers prefer strong flavor and sweetness of fresh and heated (boiled or grilled), besides soft texture of grilled bunching onion. Preferences on pungency, stickiness and texture for fresh, boiled and grilled spring onion vary depending on the consumer. Japanese food restaurants tend to value taste and flavor, while other types of restaurants tend to consider price and spring onion size.

Interspecies hybridization plays an important role in onion breeding as it allows introgression of valuable traits with improved characteristics. Among the interspecific crosses in *Allium*, those between *A. fistulosum* and *A. cepa* have been studied extensively because the former represents a rich source of several agronomic traits including resistance to diseases and pests (Budylin et al. 2014; Kik 2002) lacking in bulb onion. Breeders have used introgression of resistant genes from *A. fistulosum* into *A. cepa* for decades with many crosses between these species achieved (Table 4.6). Some commercial interspecific hybrids obtained are Beltsville Bunching which is an amphidiploid species of *A. cepa* and *A. fistulosum*, Delta Giant (backcross of *A. cepa* var. *ascalonicum* with an amphidiploid of shallot × *A. fistulosum*),

**Table 4.6** Interspecific crosses involving *A. fistulosum*

| Cross | References |
|---|---|
| *A. cepa* × *A. fistulosum* | Levan (2010), Saini and David (1967), El-Gadi and Elkington (1975), Dolezel et al. (1980), Peffley and Mangum (1990), Ulloa et al. (1994), and Kudryavtseva et al. (2019) |
| *A. fistulosum* × *A. ascalonicum* | Cochran (1950) and Arifin and Okudo (1996) |
| *A. fistulosum* × *A.cepa* | Dolezel et al. (1980), Peters et al. (1984), Corgan and Peffley (1986), Song et al. (1997), and Kudryavtseva et al. (2019) |
| *A. fistulosum* × *A. galanthum* | El-Gadi and Elkington (1975) |
| *A. fistulosum* × *A. royeli* | McCollum (1982) |
| *A. fistulosum* × *A. schoenoprasum* | Umehara et al. (2006a) |

Top Onion which is a diploid interspecific between *A. cepa* × *A. fistulosum* and Wakegi Onion which is a diploid interspecific between shallot × *A. fistulosum* (Kik 2002).

Studies on interspecific hybridization between *Allium cepa* and *A. fistulosum*, and research has continued over the years (Chuda and Adamus 2012). However, low fertility of the interspecific $F_1$ hybrid between these two species has made progress rather slow. Van der Valk et al. (1991) suggested that strong pre-fertilization and post-fertilization barriers limit the recombination between the chromosomes of bulb and bunching onions. Ulloa et al. (1994), showed that the number of bridges and fragments varied between the $F_1$ hybrid of *A. fistulosum* × *A. cepa* and $BC_1$ progenies (*A. fistulosum* × *A. cepa*) × (*A. cepa*). The $F_1$ hybrid and all $BC_1$ progenies were either sterile or very low seed set. Development of addition lines (Peffley et al. 1985) and the use of *A. roylei* as a bridging species (Khrustaleva and Kik 1998) was proposed to increase the possibility of genetic introgression from *A. fistulosum* into *A. cepa* (Martinez et al. 2005).

Since the 1980s, $F_1$ hybrids cultivars have been released in Japan with 80% new cultivars released per year. There are over 120 registered cultivars of spring onion in Japan with some developed using male sterility. Shigyo et al. (1996) established a series of alien addition lines, representing the 8 different chromosomes of shallot (*Allium cepa* Aggregatum group) in an *A. fistulosum* background. Yamashita et al. (1999) suggested that a male sterile line of *A. fistulosum* developed by continuous backcrossing with *A. galanthum* could be useful for spring onion breeding to eliminate the emasculation process and to produce large numbers of $F_1$ seeds. In Japanese bunching onion, male sterility is controlled by the interaction between a cytoplasmic factor *S*, and two nuclear genes $Ms_1$ and $Ms_2$ (Moue and Uehara 1985) with male sterility occurring when it is homozygous recessive (Havey 2002). The genotype of the male sterile plants and their maintainer are $Sms_1ms_1ms_2ms_2$ and $Nms_1ms_1ms_2ms_2$, respectively (Inden and Asahira 1990). Cytoplasmic male sterility (CMS) is

important in spring onion breeding as it allows easy propagation of male sterile plants using an appropriate maintainer line (Havey 2004; Yamashita et al. 2009). However, the CMS expression depends on the genetic relationship between the cytoplasm donor species and bunching onion. Yamashita et al. (2009) reported that the frequency of male sterile plants in 8 of 135 spring onion accessions from Japan, China, Mongolia, Korea and Taiwan varied from 1.7% to 24.5%.

Kudryavtseva et al. (2019) revealed that spontaneous chromosome duplication produced allotetraploids from interspecific hybrids between *Allium cepa* and *A. fistulosum* by using GISH analyses. Interspecific hybrids between spring onion (*A. fistulosum*) and chive (*A. schoenoprasum)* by reciprocal crossing through ovary culture producing vigorous growth and higher edible parts than their parents when using *A. fistulosum* as the seed plant (Umehara et al. 2006b). Yamasaki et al. (2011) reported that 24 h photoperiod treatment increased plant height and leaf number in both Kincho and Choetsu cvs. However, 16 h photoperiod only increased the plant height and leaf sheath diameter of cv. Choetsu but did affect cv. Kincho. Meanwhile, 16% of treated plants bolted under 24 h, 54% under 16 h and 77% under 8 h in cv. Kincho for 30 days of treatment. More than 30 days of treatment increased the percentage of bolting plants for 16 h and 24 h photoperiods. However, no differences in percentage of bolting were observed in the 60-day treatment for the 3 photoperiods (8, 16, 24 h) tested. Long-day photoperiod delayed bolting and increased the number of leaves in both cultivars. They suggested that spring onion requires facultative short-day during flower initiation process and that the inhibition of flower initiation by a long photoperiod can be overcome at low temperature.

### *4.6.2 Molecular Breeding*

Spring onion is second most important species in the genus *Allium* because of its disease resistance, ecological adaptability and close relationship to *A. cepa*. Alien chromosome addition lines from *A. fistulosum* could enhance the possibility of breeding for selective chemical components in *A. cepa* (Yaguchi et al. 2009). Breeding of spring onion is time-consuming and requires a large area. It takes many years, usually 1–2 years per generation, because of slow plant growth. Development of molecular markers is important in facilitating the establishment of a genetic basis for plant breeding. Quantitative trait loci (QTL) analysis based on genetic linkage maps is required to reveal the mode of inheritance of selective agronomic traits targeted for crop improvement. Several studies on the use of molecular markers for the development of linkage map and QTL analysis are reported (Table 4.7). Yamashita et al. (1999) published the use of isozymes and RAPD analysis to develop genetic markers linked to the fertility restoring gene (*Rf*) for cytoplasmic male sterility (CMS) in backcross progenies between *A. galanthum* and *A. fistulosum*. Sequence characterized amplified region (SCAR) markers linked to fertility restoring gene for CMS were reported by Yamashita et al. (2002) and could be applied for marker associated selection (MAS).

**Table 4.7** Molecular markers used for the development of linkage map and QTL analysis in *Allium fistulosum*

| Marker type | Purpose | Country | References |
|---|---|---|---|
| Random-amplified polymorphic DNA (RAPD) | Study of gene for CMS of *A. fistulosum* | Japan | Yamashita et al. (1999) |
| Random-amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) | Study of fertility restoring gene for CMS in *A. fistulosum* | Japan | Yamashita et al. (2002) |
| Simple sequence repeat (SSR) | Organization of SSR in heterochromation of *A. fistulosum* | Russia | Fesenko et al. (2002) |
| Simple sequence repeat (SSR) | Development of microsatellite marker in *A. fistulosum* | Japan, China | Song et al. (2004), Tsukazaki et al. (2007), and Yang et al. (2015) |
| Amplified fragment length polymorphism (AFLP) | Relationship between heterosis and genetic distance in *A. fistulosum* | Japan | Ohara et al. (2005b) |
| Amplified fragment length polymorphism (AFLP), cleaved amplified polymorphic sequence (CAPS) and simple sequence repeat (SSR) | Genetic linkage map of *A. fistulosum* | Japan, Korea | Ohara et al. (2005a) |
| Random-amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequence (CAPS) | To confirm the hybridity between *A. fistulosum* × *A. schoenoprasum* | Japan | Umehara et al. (2006b) |
| Simple sequence repeat (SSR) | Construction of SSR-based chromosome map in *A. fistulosum* | Japan | Tsukazaki et al. (2008) |
| Simple sequence repeat (SSR) and cleaved amplified polymorphic sequence (CAPS) | Mapping QTL controlling seedling growth in *A. fistulosum* | Japan | Ohara et al. (2009) |
| Simple sequence repeat (SSR) | Identification of alien chromosome additions from *A. fistulosom* in shallot | Japan & Vietnam | Yaguchi et al. (2009) |
| Random-amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) | Cytoplasmic male sterility (CMS) analysis in *Allium* | China | Gai and Meng (2010) and Gao et al. (2013, 2015) |
| Simple sequence repeat (SSR) | Classification of *A. fistulosum* varieties | Japan | Tsukazaki et al. (2010) |
| Expressed sequence Tag (EST)- simple sequence repeat (SSR), simple sequence repeat (SSR), single nucleotide polymorphisms (SNP) | Comparative genomics resource for *Allium* | Japan, New Zealand, Netherlands | McCallum et al. (2012) |

(continued)

**Table 4.7** (continued)

| Marker type | Purpose | Country | References |
|---|---|---|---|
| Random-amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), sequence-tagged site (STS), expressed sequence tag (EST) | QTL analysis for pseudostem pungency in *A. fistulosum* | Japan | Tsukazaki et al. (2012) |
| Amplified fragment length polymorphism (AFLP) and sequence characterized amplified region (SCAR) | Development of SCAR marker for CMS | China | Wang et al. (2013) |
| Simple sequence repeat (SSR) and ribonucleic acid sequencing (RNAseq) | Study of cuticular wax-related genes in *A. fistulosum* | China | Liu et al. (2014) |
| Simple sequence repeat (SSR) and single nucleotide polymorphisms (SNP) | Genetic linkage map of *A. fistulosum* | Japan | Tsukazaki et al. (2015) |
| Single nucleotide polymorphisms (SNP) | SNP markers for introgression breeding in onion | Netherlands | Scholten et al. (2016) |
| Simple sequence repeat (SSR) and expressed sequence tag (EST) | QTL for bolting time in *A. fistulosum* | Japan | Wako (2016) |
| Simple sequence repeat (SSR) | Association of tandem repeat to major chromosomal landmarks in *A. fistulosum* | Russia, Belgium | Kirov et al. (2017) |
| Single nucleotide polymorphisms (SNP) | Development of KASP marker for CMS | China | Gao et al. (2018) |
| Single nucleotide polymorphisms (SNP) | Varietal identification of open-pollinated onion cultivars | Korea | Lee et al. (2018) |
| Mitochondrial genome sequences | Development of molecular markers distinguishing between *A. cepa* and *A. fistulosum* | Korea | Kim and Kim (2019) |
| SSR and ISSR | Genetic diversity of *A. fistulosum* | Nigeria | Abutu et al. (2020) |

Organization of a 378-bp satellite repeat in terminal heterochromatin of *Allium fistulosum* was reported by Fesenko et al. (2002). Isolation and development of SSR markers in spring onion as a primary basis for breeding was described by Song et al. (2004) and Yang et al. (2015). A total of 1796 SSR clones from SSR-enriched DNA libraries of spring onion were identified by (Tsukazaki et al. 2007) which are applicable for phylogenic analysis and construction of genetic linkage maps. Ohara et al. (2005b) used AFLP markers to study the relationship between heterosis and genetic distance in intervarietal $F_1$ hybrids of *A. fistulosum*. Tsukazaki et al. (2008) isolated 1940 SSR clones from size-fractionated genomic DNA libraries with potential use

for cultivar identification and hybrid purity identification. A genetic map based on single nucleotide polymorphisms (SNPs) markers was assembled by using interspecific $F_1$ hybrid between *A. roylei* × *A. fistulosum* (Scholten et al. 2017).

The first linkage map of spring onion with AFLPs was constructed by Ohara et al. (2005a) using reciprocally backcrossed progenies of two inbred lines of Japanese bunching onion (*A. fistulosum*) based on 149 AFLP markers, 1 cleaved amplified polymorphic sequence (CAPS) and 13 SSRs using reciprocally backcrossed progenies. Tsukazaki et al. (2008) constructed a detailed linkage map consisting of 17 linkage groups with 212 bunching onion SSR markers and 42 bulb onion (*A. cepa*) SSR, InDel, CAPS or dCAPS markers, covering 2069 cM. Ohara et al. (2009) added 24 SSRs and 1 CAPS to the previous map developed by Ohara et al. (2005a) on reciprocally backcrossed progenies resulting into 16 linkage group for the J map and 15 linkage groups for the D map. QTL analyses conducted showed that the seedling growth is controlled by many QTLs that exhibit various modes of gene actions, additive, dominant and overdominant.

Classification and identification of 30 bunching onion varieties based on simple sequence repeat (SSR) markers was reported by (Tsukazaki et al. 2010). Numerous DNA markers based on SSRs, SNPs and InDels were developed (Tsukazaki et al. 2015). Identification of restorer genes involved in the restoration of fertility in CMS is essential for the establishment of molecular breeding system. RAPD and SCAR marker distinguishing between *N* and *S* cytoplasm in several spring onion cultivars were reported by Gai and Meng (2010) and Gao et al. (2013, 2015). SCAR marker developed from AFLP marker distinguishing between CMS and fertile lines could be used by breeder to extract maintainer lines from open-pollinated spring onion (Wang et al. 2013). Gao et al. (2018) reported a competitive allele-specific PCR (KASP) marker developed from SNP detected in the *atp6* gene could discriminate between male-sterile and normal cytoplasmic types.

Sequencing a huge genome by the high-throughput method has been initiated, as well as the development of molecular markers and linkage mapping. Allium Map for cultivated *Allium* vegetable including spring onion was developed by McCallum et al. (2012) and provides a genetic map and marker data from multiple *Allium* species. High throughput SNP genotyping, functional genomics using RNAi and transcriptome mapping can be exploited to understand the function of genes in the genome. Transcriptome sequencing utilizing next-generation sequencing (NGS) has become an effective tool to discover novel genes in several crops. A total of 798 genes, representing 1.86% of total putative unigenes, were differentially expressed between waxy spring onion and non-waxy mutant spring onion varieties (Liu et al. 2014). More than 50,000 unigenes were obtained from transcriptome shotgun assembly (TSA) of next-generation sequencing (NGS) data.

Genomic SSRs from bunching onion and EST-SSRs from bulb onion were used by Wako (2016) to study the genetic basis of bolting time in bunching onion. They reported that 2 QTLs associated with bolting time were detected on the linkage groups of chromosomes 1 (Chr. 1a) and chromosome 2 (Chr. 2a). However, the QTL on Chr. 1a was not detected in the KiC population (inbred line from ever-flowering, Kitanegi and late-bolting, Cho-etsu) grown in a heated greenhouse under

unvernalized conditions. A single QTL with major effect was identified exclusively on the linkage group Chr. 2a in the SaT03 population (cross between early-bolting line, Chuukanbohon Nou 1 and late-bolting, Fuyuwarabe) were evaluated under field conditions. Tsukazaki et al. (2017) reported 27 QTLs for the 6 morphological traits (plant height, leaf length, pseudostem length, leaf width, pseudostem width, number of leaf sheath) in 16 regions of 11 linkage groups, with a major QTL for the number of leaf sheaths repeatedly detected on Chr. 8. They discovered 2 QTLs associated with pseudostem pigmentation on linkage groups Chr. 4a and Chr. 5a-2. Tsukazaki et al. (2012) revealed a major QTL for pseudostem pungency located within a 24.2 cM interval on Chr. 2a of spring onion.

A SSR-tagged breeding scheme to enhance the rapidity, ease and accuracy of variety identification and $F_1$ purity test was suggested by Tsukazaki et al. (2006). Tsukazaki et al. (2009) later proposed to protect breeders' rights and confer traceability in allogamous crops. The scheme consists of three steps (Fig. 4.3):

(a) Selection of a small number of highly polymorphic SSR loci that are not tightly linked to each other. Selection of prevailing allele at each locus, since the parental lines of the $F_1$ hybrids must carry different alleles at each selected locus.
(b) Selection of plants in a foundation seed field that are homozygous for the prevailing allele at all the SSR loci selected.
(c) Harvest of foundation seed from the plants selected. For $F_1$ breeding, one parental line should be homozygous at each selected SSR locus and the other should be homozygous for another allele.

Production of stock seed normally will be followed by marketing of seed. Open-pollinated varieties should be homozygous and uniform at the selected SSR loci while the $F_1$ varieties should be uniformly heterozygous. Open-pollinated varieties and the parental lines of the $F_1$ varieties should not exhibit inbreeding depression since most of the loci can maintain their original heterogeneity.

### *4.6.3 Tissue Culture Applications*

Micropropagation of *Allium fistulosum* can avoid the maintenance of male sterile lines by a non-restorer fertility line. A large number of identical offspring from a limited amount of parent material could be obtained to increase the number of female plants for the production of $F_1$ hybrid seed. Song and Peffley (1994) reported on in vitro culture of *A. fistulosum* and *A. cepa* interspecific derivatives (Table 4.8). Furthermore, the high proliferation of adventitious shoots were obtained from shoot tip culture of spring onion using MS medium (Murashige and Skoog 1962) supplemented with 2 mg/l 6-furfurylaminopurine (kinetin) and 0.5 mg/l 1-naphthylacetic acid (NAA) at 20 °C. Fujieda et al. (1977) found that in the presence of cytokine it stimulated the multiplication of adventitious shoots, but completely inhibited adventitious roots.

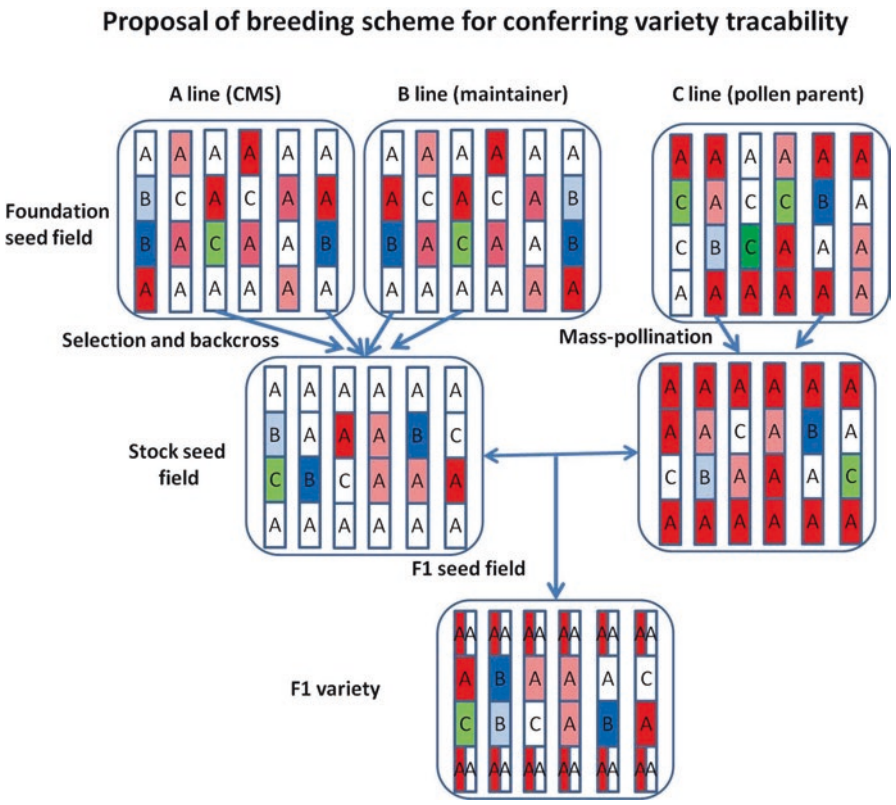


**Fig. 4.3** The SSR-tagged breeding scheme for conferring variety traceability

Organ culture such as embryo and ovary can facilitate the recovery of progeny from high sterile interspecific $F_1$ hybrids and backcross progeny to overcome incompatibility in interspecific hybrids. Regeneration into haploid plantlets has been achieved in spring onion using flower and ovary culture (Ibrahim et al. 2016). Hybrid plants were obtained from embryo cultured on B5 medium (Gamborg et al. 1968) without growth regulators (Peffley 1992). Gonzales and Ford-Lloyd (1987) demonstrated successful embryo rescue of hybrids from crosses between *Allium cepa* and *A. fistulosum* using B5 medium. Umehara et al. (2006b) reported the production of interspecific hybrids between *A. fistulosom* × *A. macrostemon* through ovary culture using B5 medium modified by Dunstan and Short (BDS) containing 30 g $l^{-1}$ sucrose. Hybrids produce from germinated embryos transferred to BDS medium containing 1 mg $l^{-1}$ 6-benzylaminopurine and 15 g $l^{-1}$ sucrose followed by subculturing on phytohormone-free B5 medium showed intermediate traits of both parents.

Somatic embryogenesis can provide regeneration of a large number of individuals from a single progenitor. However, the occurrence of somaclonal variation can

**Table 4.8** Summary of tissue culture studies on *Allium fistulosum*, A. fistulosum × A. cepa and A. cepa × A. fistulosum.

| Species | Type of tissue culture | References |
|---|---|---|
| *A. fistulosum* | Callus | Lin and Cui (1982), Tashiro et al (1985), and Kim and Soh (1996) |
| | Callus and embryo | Van der Valk et al (1992) and Phillips and Hubstenberger (1987) |
| | Ovary and ovule | Ibrahim et al. (2016) |
| *A. fistulosum* × *A. cepa* | Callus | Peffiey (1992) and Phillips and Hubstensberger (1987) |
| | Embryo | Lu et al. (1989) and Van der Valk et al. (1991) |
| | Somatic hybrid | Shimonaka et al. (2002) |
| *A. cepa* × *A. fistulosum* | Callus | Shahin and Kaneko (1986) |
| | Embryo | Gonzalez and Ford-Lloyd (1987) |
| | Micropropagation | Mar'yakhina et al. (1983) |
| | Ovary and ovule | Guan and Peffley (1989) |
| *A. fistulosum* × *A. macrostemon* | Ovary | Umehara et al. (2006b) |

be an issue in determining desired clones. Mutations and polyploidization are quite frequent in callus culture and thus enables their possible use in breeding program using somaclonal variation (Inden and Asahira 1990). Plant regeneration from callus culture is well established in *Allium fistulosum* and interspecific hybrids between alliums. Phillips and Hubstenberger (1987) developed procedures for micropropagation and plant regeneration from callus for *A. fistulosum, A. altaicum, A. galanthum, A. roylei* and selected progeny of interspecific crosses of *A. cepa* × *A. fistulosum, A. cepa* × *A. galanthum* and *A. cepa* × *A. oschaninii*. Lu et al. (1989) reported high frequency somatic embryo production from callus culture using BDS media supplemented with moderate to high auxin level and 2.5 g $l^{-1}$ proline and Shahin's vitamins. A reproducible protoplast culture system using BDS basal medium supplemented with 5mM potassium nitrate, 2 μM 2,4,dichlorophenoxyacetic acid (2,4,-D) and 0.2 or 1 μM 6-benzylaminopurine (BAP) and a combination of 0.2 M sucrose and 0.2 M glucose has been established by Shimonaka et al. (2001). Plantlet regeneration was achieved 2–3 months after inoculating protoplast-derived calli to a half N MS medium. This finding enables the introduction of targeted genes such as disease resistance or male sterile genes from *Allium* species into spring onion using cell fusion or electroporation. Interspecific somatic hybrids between *A. fistulosum* and *A. cepa* are reported by Shimonaka et al. (2002) using protoplast electrofusion. Several amphidiploids and alloplasmic plants which are valuable for spring onion breeding have been produced.

The term *haploid* refers to a plant which possesses the gametophytic number of chromosomes in their sporophytes. Monoploids contain a single genome from

diploid species while polyhaploids containing two or more genomes are derived from polyploid species. Haploid plants will become doubled-haploids (DHs) following chromosome doubling. This doubled-haploid methodology offers several advantages to plant improvement programs as it provide a rapid approach to achieve homozygosity. Since haploid plants carry only one set of alleles at each locus, upon doubling, homozygous and homogeneous lines are available. This allows identification of superior parental combinations, evaluation of environment × genotype interactions, avoids masking of recessive genes, and evaluation of qualitative and quantitative traits (Snape 1988).

Ibrahim et al. (2016) developed frequency of embryogenesis in spring onion using flower and ovary culture using cultured in BDS medium supplemented with 2 mg $l^{-1}$ 2,4-D and 2 mg $l^{-1}$ BAP fortified with 100 g/l sucrose, 200 mg $l^{-1}$ proline and 500 mg $l^{-1}$ myo-inisitol. Calli were recorded around 90 days after ovary inoculation and shoot induction was observed after 60 days of callus induction in BDS media (Dunstan and Short 1977). The cultures were green in color during the first 3 months and gradually turned yellowish as the culture progressed. Calli from the ovule were easily recognized with ovary burst after 4–5 months of inoculation (Fig. 4.4).

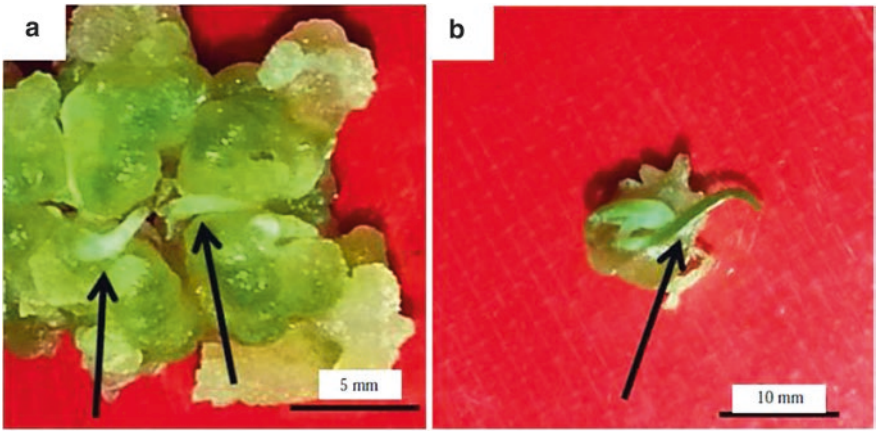


**Fig. 4.4** (**a**) Shoot formation has been observed from callus inoculated from the ovary culture in BDS media, (**b**) Shoot regeneration has been observed after 150 days of culture. (Source: Ibrahim et al. 2016)

## 4.7 Conclusions and Prospects

There are seven major edible species among the hundreds of *Allium* varieties available worldwide. Great adaptability to varied climatic conditions has created many different varieties of the crop. Each cultivar has a commercial characteristic value due to usage including in food and Chinese medicine. Different studies show spring onion has excellent antibacterial and antifungal properties. Some species also purportedly show anti-platelet, antioxidative, anti-hypertensive, and anti-hyperlipidemic properties, and reduction of cholesterol level which may decrease the risk of heart attack. The plants are rich source of vitamin C, A and $B_6$, thiamine, folate and minerals.

Spring onion can be grown in most types of soil but in recent years several countries have reported different diseases on spring onion due to continuous cultivation. Japan and Taiwan are good examples for great adaptability of the crop, where intense cultivation is combined with selection, breeding and the development of a good marketing network which has led to greatly increased production; this indicates that there is great scope for the development of better cultivars and increased commercialization and intensification of production in Southeast Asia.

Several new methods developed to achieve high yields and disease resistance in spring onion; organ culture and somatic embryogenesis will help to retain the $F_1$ progeny and produce large single progenitor respectively. Recent efforts in developing high yielding cultivars with specific and broad adaptability, precocity, resistance to various pest and diseases, and good marketing quality have been initiated, and there is a great need to use biotechnological approaches for the development of new cultivars with high yield and better disease resistant characteristics.

## Appendix I: Research Institutes Relevant to Spring Onion

| Institution name | Specialization and research activities | Address | Contact information and website |
|---|---|---|---|
| Institute for Horticultural Development, Agriculture Victoria | Study on growth and dry matter production of spring onion, white rot disease management | Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia | daryl.joyce@nrevic.gov.au; https://www.ausvegvic.com.au/pdf/r%26d_VG01096_white_rot_onion_integrated_control_strategy_booklet.pdf |
| University of Queensland | Disease forecasting system in spring onion | Queensland 4343, Australia | v.galea@uq.edu.au https://researchers.uq.edu.au/researcher/200 |
| Department of Genetics and Plant Breeding, Bangladesh Agricultural University | SNP markers, molecular breeding | Mymensingh 2202, Bangladesh | sathisbioinfo@gmail.com https://www.mdpi.com/2073-4395/8/9/179 |
| Center of Medical Genetics, Ghent University | Cytogenetics, molecular markers, spring onion breeding | Ghent, Belgium | kirovez@gmail.com https://pubmed.ncbi.nlm.nih.gov/28150039/ |
| Instituto Federal Catarinense | Crop improvement | C. Postal 441, 89163-356 Rio do Sul-SC, Brazil | marcuzzo@ifc-riodosul.edu.br; https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-05362014000300363 |
| University of Saskatchewan | Phylogenetics and taxonomy of *Allium* | Saskatoon, Canada | hugo.cota@usask.ca https://www.researchgate.net/publication/263500245_A_taxonomic_revision_of_Allium_Alliaceae_in_the_Canadian_prairie_provinces |
| Kunming Institute of Botany, Chinese Academy of Sciences | Study of chloroplast genome of *A. fistulosum* | Kunming, China | sunhang@mail.kib.ac.cn; https://www.tandfonline.com/doi/pdf/10.1080/23802359.2018.1545532 |

| | | | |
|---|---|---|---|
| Beijing Technology and Business University (BTBU) | Nutritional value of *A. fistulosum* | Beijing 100048, China | chenht@th.btbu.edu.cn https://europepmc.org/article/med/31108752 |
| Beijing Academy of Agriculture and Forestry Science (North China) | Genetics and breeding of spring onion, transcriptome sequencing | Beijing 100097, China | wangyongqin@nercv.org; https://web.b.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=16616596&AN=117067465&h=rpJH2%2fnRPYhGXzYAIQ5jM6HOBvVg%2fFbcqJHYXlaSayCx34ik0ZiFQYjpy2GM%2fi%2bbH0nL%2fuL%2bpCRml%2bFebRMIQA%3d%3d&crl=c&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3fdirect%3dtrue%26profile%3dehost%26scope%3dsite%26authtype%3dcrawler%26jrnl%3d16616596%26AN%3d117067465 |
| University of Chinese Academy of Science | Genomic study of *A. fistulosum* | Beijing, China | sunhang@mail.kib.ac.cn https://search.proquest.com/openview/03b13ee11d15e8b54850a2f6dd17e8b8/1?pq-origsite=gscholar&cbl=3933403 |
| College of Horticulture and Gardening, Yangtze University | SSR markers, RNA sequencing, molecular markers | Jingzhou, China | lchliu18@yangtzeu.edu.cn https://pdfs.semanticscholar.org/b581/67fcada93d4f194e6f5a0d8a834632f038ff.pdf |
| College of Horticulture, Northwest A&F University | Physiology of *A. fistulosum* | Yangling, Shaanxi 712100, People's Republic of China | chengzh@nwsuaf.edu.cn https://pubmed.ncbi.nlm.nih.gov/24199907/ |
| College of Resources and Environmental Sciences, China Agricultural University | Effect of mycorrhizal colonization on pungency | Beijing, China 100094 | junlingz@cau.edu.cn https://pureadmin.qub.ac.uk/ws/files/452805/GuoT%20et%20al%202007.pdf |
| College of Horticulture, Shandong Agricultural University | SSR markers, transcriptome, molecular markers, molecular breeding, cytoplasmic male sterility | Tai'an 271018, P.R. China | sqliu@sdu.edu.cn https://www.mdpi.com/journal/ijms/special_issues/plant-molecular-biology?view=abstract&listby=type |

(continued)

| Institution name | Specialization and research activities | Address | Contact information and website |
|---|---|---|---|
| Institute of Medical Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College | Physiology of spring onion | Beijing 100193, China | qianwangcau@126.com https://www.cabdirect.org/cabdirect/abstract/20143400467 |
| Yantai Agricultural Science Academy of Shandong Province | Effect of spring onion root against root knot nematodes | Yantai, Shandong, P.R.China | ytnkyscs@163.com https://journals.plos.org/plosone/article/authors?id=10.1371/journal.pone.0201471 |
| The First Hospital of China Medical University | Medicinal value of *Allium* | Shenyang 110001, China | zli@cmu.edu.cn; https://onlinelibrary.wiley.com/doi/abs/10.1111/ajco.13133 |
| Shanghai Jiao Tong University | Supply and demand of spring onion | Shanghai, People's Republic of China | gengna@sjtu.edu.cn; |
| Dept of Food Science and Technology, Chia Nan University of Pharmacy and Science | Nutritional and medicinal value of spring onion | 60 Erh-Jen Road, Section 1, Pao-An, Jen-te Hsiang, Tainan Hsien, Taiwan | ipdduh@mail.chna.edu.tw |
| Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Ministry of Agriculture | SSR markers, RNA sequencing, molecular breeding | Beijing 100097, China | wangyongqin@nercv.org |
| Beijing Academy of Agriculture and Forestry Science | Effect on nitrogen and sulfur on the growth of spring onion | Beijing, China | wangyongqin@nercv.org https://www.researchgate.net/publication/269356745_Effect_of_Nitrogen_and_Sulphur_on_the_Growth_and_Qualities_of_Bunching_Onion |

| | | | |
|---|---|---|---|
| Yantai Agricultural Science Academy of Shandong Province | Nematodes control in spring onion | Yantai, Shandong, P.R.China | ytnkyscs@163.com https://www.researchgate.net/publication/326703074_Inhibitory_effects_of_components_from_root_exudates_of_Welsh_onion_against_root_knot_nematodes |
| Shanghai Jiao Tong University | Seed demand forecasting of spring onion | China | gengna@sjtu.edu.cn; https://journals.plos.org/plosone/article/authors?id=10.1371/journal.pone.0219889 |
| Colombian Corporation for Agricultural Research (Corpoica) | Agronomic Evaluation of Bunching Onion in the Colombian Cundiboyacense High Plateau | CI Palmira, Colombia and CI Tibaitatá, Mosquera, Colombia | galeanomendoza@gmail.com https://core.ac.uk/download/pdf/205389639.pdf |
| Department of Food Science, Yuanpei University | Nutritional and medicinal value of *Allium* | Hsinchu, Chinese Taipei | hungder@mail.ypu.edu.tw, https://www.scirp.org/html/22-2700684_35298.htm |
| Department of Plant Medicine, National Chiayi University | Molecular breeding, crop improvement | 60004, Taiwan | chienjui.huang@mail.ncyu.edu.tw; https://web.b.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=11254653&AN=130550885&h=v9HLDeIM%2fFJLxFvToiyvNQAzGyQbJykSwJ%2btnfTxbaKX7SScpNpWq0Un7BNWHLaetSGxWGqKfHD5CtN2Utoy6A%3d%3d&crl=c&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3fdirect%3dtrue%26profile%3dehost%26scope%3dsite%26authtype%3dcrawler%26jrnl%3d11254653%26AN%3d130550885 |
| Université Paris-Sud | *Allium* genomic study | CNRS UMR 8079, Bâtiment 360, 91405 Orsay, France | agnes.richroch@esv.u-psud.fr https://www.sciencedirect.com/science/article/abs/pii/S0378111997003958 |

(continued)

| Institution name | Specialization and research activities | Address | Contact information and website |
|---|---|---|---|
| Botanical Garden of the University of Osnabrück | Phylogenetics, molecular markers | Albrechtstr 29, 49076 Osnabrück, Germany | nfriesen@uni-osnabrueck.de https://pubmed.ncbi.nlm.nih.gov/26639102/ |
| Leibniz Institute of Vegetable and Ornamental Crops | Physiology of spring onion | Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany | perner@igzev.de https://pubmed.ncbi.nlm.nih.gov/18457399/ |
| Institute of Plant Genetics and Crop Plant Research (IPK) | RAPD and non-coding chloroplast DNA study, cytogenetics, molecular markers | Correnssstr 3, 06466 Gatersleben, Germany | rutten@ipk-gatersleben.de |
| Dept. of Horticulture, University of Georgia | Physiology of onion | 1111 Miller Plant Science Bldg., Athens, GA 30602 | gboyhan@uga.edu |
| Indian Institute of Horticultural Research | Molecular markers, *Allium* genome and breeding | Bangalore, India | dclreddy@gmail.com |
| University of Bari 'Aldo Moro' | Physiology of spring onion | Valenzano, Bari, Italy | mariano.fracchiolla@uniba.it |
| National Institute of Vegetable and Tea Science (NIVTS), National Agriculture and Food Research Organization (NARO) | Microsatellite, SSR, AFLP and molecular markers, genetic mapping, QTL study, molecular breeding | 360 Ano-Kusawa, Tsu, Mie 514-2392, Japan | tsuka@affrc.go.jp |
| National Agricultural Research Center for Tohoku Region (NARCT) | SSR markers, molecular breeding, crop improvement | 4 Akahira, Shimo-kuriyagawa, Morioka, Iwate 020-0198, Japan | amhonjo@affrc.go.jp |
| Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF) | SSR markers, molecular breeding | 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan | kan@staff.or.jp |

| | | | |
|---|---|---|---|
| Tohoku Agricultural Research Center, National Agriculture and Food Research Organization (NARO) | QTL study, molecular breeding | 92 Nabeyashiki, Shimo-kugiyagawa, Morioka, Iwate 020-0123, Japan | tsuka@affrc.go.jp |
| National Research Institute of Vegetables, Ornamental Plants and Tea | Physiology of *A. fistulosum*, molecular markers, QTL mapping, molecular breeding | Kurume, Fukuoka 839-8503, Japan | yamasaki@narc.affrc.go.jp |
| Western Region Agricultural Center NARO | QTL analysis, molecular breeding | 1-3-1 Senyu-cho, Zentsuji, Kagawa 765-8508, Japan | kenyamas@affrc.go.jp |
| Osaka City University | Study on antioxidant activity and flavonoid content | Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan | yamamoto@life.osaka-cu.ac.jp |
| Institute of Plant Genetics and Crop Plant Research (IPK) | Phylogenetics, molecular markers, molecular breeding | Corrensstr 3, 06466 Gatersleben, Germany | blattner@ipk-gatersleben.de |
| Botanical Garden of the University of Osnabrück | Phylogenetics, molecular markers | Albrechtstr 29, 49076 Osnabrück, Germany | nfriesen@uni-osnabrueck.de |
| Plant Nutrition, Institute of Crop Science, Humboldt University Berlin | Effect of fertilizer on yield of spring onion | nvalidenstr. 42, 10115 Berlin, Germany | george@igzev.de |
| Leibniz Institute of Vegetable and Ornamental Crops | Physiology of spring onion | Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany | perner@igzev.de |
| Institute of Plant Genetics and Crop Plant Research (IPK) | RAPD and non-coding chloroplast DNA study, cytogenetics, molecular markers | Corrensstr 3, 06466 Gatersleben, Germany | rutten@ipk-gatersleben.de |
| Dept. of Horticulture, University of Georgia | Physiology of onion | 1111 Miller Plant Science Bldg., Athens, GA 30602 | gboyhan@uga.edu |

(continued)

| Institution name | Specialization and research activities | Address | Contact information and website |
|---|---|---|---|
| Indian Institute of Horticultural Research | Molecular markers, *Allium* genome and breeding | Bangalore, India | dclreddy@gmail.com |
| University of Bari 'Aldo Moro' | Physiology of spring onion | Valenzano, Bari, Italy | mariano.fracchiolla@uniba.it |
| National Agricultural Research Center for Tohoku Region (NARCT) | SSR markers, molecular breeding, crop improvement | 4 Akahira, Shimo-kuriyagawa, Morioka, Iwate 020-0198, Japan | amhonjo@affrc.go.jp |
| Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF) | SSR markers, molecular breeding | 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan | kan@staff.or.jp; i-kono@staff.or.jp |
| Tohoku Agricultural Research Center, National Agriculture and Food Research Organization (NARO) | QTL study, molecular breeding | 92 Nabeyashiki, Shimo-kugiyagawa, Morioka, Iwate 020-0123, Japan | tsuka@affrc.go.jp |
| National Research Institute of Vegetables, Ornamental Plants and Tea | Physiology of *A. fistulosum*, molecular markers, QTL mapping, molecular breeding | Kurume, Fukuoka 839-8503, Japan | yamasaki@narc.affrc.go.jp |
| Western Region Agricultural Center, NARO | QTL analysis, molecular breeding | 1-3-1 Senyu-cho, Zentsuji, Kagawa 765-8508, Japan | kenyamas@affrc.go.jp |
| Osaka City University | Study on antioxidant activity and flavonoid content | Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan | yamamoto@life.osaka-cu.ac.jp |
| Tohuku University | Biochemical characterization in *A. fistulosum* | Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan | koji.muramoto.d5@tohoku.ac.jp |

| | | | |
|---|---|---|---|
| Faculty of Agriculture, Tottori University | Micropropagation of spring onion, cytogenetics, microsatellite markers in *Allium* | 4-101 Koyama-Minami, Tottori 680-8553, Japan | itai@muses.tottori-u.ac.jp |
| Faculty of Agriculture, Saga University | Isozymes markers, molecular markers in *Allium,* onion breeding | Saga 840-8502, Japan | tashiroy@cc.saga-u.ac.jp |
| Faculty of Agriculture, Yamagata University | Mycorrhizal colonization in *A. fistulosum* | Tsuruoka 997-8555, Japan | tawaraya@tds1.tr.yamagata-u.ac.jp |
| Faculty of Agriculture, Hokkaido University | Mycorrhizal colonization in *A. fistulosum* | Sapporo, 060-8589, Japan | tatsu@agr.nagoya-u.ac.jp |
| Faculty of Agriculture, Kyushu University | Biological control, pest management | 46-01, Fukuoka 812, Japan | ueno@grt.kyushu-u.ac.jp |
| Faculty of Agriculture, Yamaguchi University | Molecular markers in *Allium,* QTL mapping, *Allium* genome study, molecular breeding | 1677-1 Yoshida, Yamaguchi 753-8515, Japan | shigyo@yamaguchi-u.ac.jp |
| Faculty of Agriculture, Kyoto Prefectural University, | Biodiversity of *Allium* | Shimogamo, Sakyo, Kyoto, Japan 606-8522 | yfujime@rio.odn.ne.jp. |
| Faculty of Life and Environmental Science, Shimane University | Micropropagation, physiology of spring onion | Matsue, Shimane 690-8504 | yano@life.shimane-u.ac.jp |
| University of Toyama | Medicinal value of *A. fistulosum* | 2630 Sugitani, Toyama, 930-0194, Japan | lee@pha.u-toyama.ac.jp |
| Faculty of Pharmaceutical Sciences, Sojo University | Medicinal value of *A. fistulosum* | 22-1, 4-Chome, Nishi-ku, Ikeda, Kumamoto, 860-0082, Japan | none@ph.sojo-u.ac.jp. |
| Kansai University of Welfare Sciences | Molecular cytogenetic, molecular breeding | Kashiwara, Osaka 582-0026, Japan | myamamoto@tamateyama.ac.jp |

(continued)

| Institution name | Specialization and research activities | Address | Contact information and website |
|---|---|---|---|
| Department of Biotechnology, Maebashi Institute of Technology | Micropropagation of spring onion | Maebashi 371-0816, Japan | ihonda@maebashi-it.ac.jp |
| Kobe Women's University | Food science | Suma-Ku, Kobe City, Japan 654-8585 | seguchi@kobe-wu.ac.jp |
| Gakushuin Women's College | Food Science | Tokyo, Japan 162-8650. | |
| Department of Pharmacognosy, Kyoto Pharmaceutical University | Food chemistry of *A. fistulosum* | Misasagi, Yamashina-ku, Kyoto 607-8412, Japan | matsuda@mb.kyoto-phu.ac.jp |
| Department of Pharmacognosy, Kyoto Pharmaceutical University | Nutritional and medicinal value of spring onion | Misasagi, Yamashina-ku, Kyoto 607-8412, Japan | matsuda@mb.kyoto-phu.ac.jp |
| Kazusa DNA Research Institute | RNA sequencing, biochemical analysis and molecular breeding | 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan | hh@kazusa.or.jp |
| Tottori Horticultural Experiment Station | Micropropagation and physiology of spring onion, rust resistance | Kurakoshi, Tottori 682-0948 | shiraiwah@pref.tottori.jp |
| Fukuoka Agricultural Research Center | Molecular marker, ovary culture | Yoshiki 587, Chikushino, Fukuoka 818-8549, Japan | umehara@farc.pref.fukuoka.jp |
| Mibyeong Research Center, Korea Institute of Oriental Medicine | Nutritional and medicinal value of spring onion | 1672 Yuseong-daero, Yuseong-gu, Daejeon 305-811, Korea | hkkim@kiom.re.kr |
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