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Present status and future opportunities in onion research: A review

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Abstract
Onions (*Allium cepa* L.) is a biennial crop of the *Alliaceae* family with edible portion of the bulbs is the enlarged leaf bases and compact stem. A wide range of landraces and cultivars harbouring immense genetic variation for different traits are available, species like *Allium cepa, A. fistulosum* and *A. roylei* can be utilized to develop cultivars resistant against different diseases along with desirable agronomic traits. To evaluate the genetic diversity in onion, number of molecular marker systems is available such as RFLP, RAPD, ISSR, SSR, CAPS and SNP but SSR markers have provided the greatest insights into onion diversity. Doubled haploid (DH) production is an alternative strategy for complete homozygosity and phenotypic uniformity to obtain inbred lines in onion along with this hybridization barrier between *A. fistulosum* and *A. cepa* can be overcome using *A. roylei* as a bridging species. Large future opportunities are there in onion research for various aspects like development of resistance varieties and hybrids to biotic and abiotic factors, improved quality standards, CMS hybrids, DH production etc.

Keywords: Present status, future opportunities, onion research

Introduction
Onions (*Allium cepa* L.) is a biennial crop of the *Alliaceae* family. The edible portions of the bulbs are the enlarged leaf bases and compact stem. Green onions, also called scallions, are eaten for their immature bulb and green foliage. Besides this, onion serves as a good medicinal compound for cataract, cardiovascular disease and cancer due to its thrombolitic and antioxidant effects as stated by Vidyavati *et al* (2010) [101]. Several antioxidant compounds, mainly polyphenols such as flavonoids and sulfur-containing compounds have been described in onion (Gorinstein *et al* 2005) [44]. Major onion producers are China, India, United States, Turkey, Japan, Spain, Holland, Poland, and Ukraine. India is the second largest producer of onion in the world with an annual output of 6.50 million tonnes from an area of 0.52 million ha (FAOSTAT, 2014) [36].

The availability of diverse germplasm is a prerequisite for the success of breeding programmes (Glaszmann *et al* 2010) [42]. The species like *Allium cepa, A. fistulosum* and *A. roylei* can be utilized to develop cultivars resistant against different diseases along with desirable agronomic traits (Vu *et al* 2012) [102]. Onions are affected by wide range of insect pests and diseases. Among them, downy mildew, fusarium basal rot, white rot, black mould, purple blotch, stemphylium blight are the diseases affecting bulb and seed crops of onion. Germplasm from different countries has been evaluated for different diseases and thrips, and a few tolerant sources identified, which can be utilized in breeding programmes (Diaz-Montano *et al* 2011) [25]. In onion, male sterility is due to interaction of cytoplasm and nuclear gene, i.e. cytoplasmic genetic male sterility (CGMS). The first male sterile plant was reported within the progenies of an onion cultivar 'Italian Red' (Jones and Emsweller 1936) [44], which was cytoplasmically inherited, and male sterility was under the control of a single recessive nuclear restorer locus (Jones and Clarke, 1943) [52]. In onion breeding, the traits such as size, shape, colour, pungency, soluble solids and disease resistance were targeted. Quantitative trait loci (QTL) analysis based on a genetic linkage map would be efficient for revealing the mode of inheritance of these traits. The first genetic linkage map of Japanese bunching onion with AFLP markers was constructed using reciprocally backcrossed progenies. The first onion genetic map was developed with the BYG15-23× AC43 F_2 population using 116 markers (RAPD and RFLP) which consists of 12 linkage groups with average distance of 9 cM. Three important characters, male sterility, bulb colour and flavour (*alliinase* activity) mapped to different chromosomes (King *et al* 1998) [68]. A herbicide resistant gene was used to develop glyphosate (Round-up) resistance onions by using *Agrobacterium* mediated transformation,

but these plants have not been released commercially (Eady *et al* 2003) [28]. RNAi suppressed the activity of lachrymatory factor synthase, resulting in a 'tear-less' onion, in which isoalliin was not converted into thiosulfinates, which is desirable compound for onion flavor and human health (Eady *et al* 2008) [33]. Although reasonable progress has been made so far in the onion and research, many important problems still have to be tackled. Rapid progress could be achieved through the use of simple *in vitro* and rapid multiplication methods.

Genetic diversity in onion

Onion has been grown from ancient times in different regions, and growers have selected desirable populations according to their needs. A wide range of landraces and cultivars harbouring immense genetic variation for different traits are available (Brewster 2008) [14]. Threat of genetic erosion due to the introduction of F_1 hybrids led to the collection of onion landraces and modern cultivars in various countries (Fritsch and Friesen 2002) [37]. These germplasm collections represent a wide range of onion gene pools and are available in various gene banks. Wild *alliums* harbour a wide range of useful traits but crossing barriers restrict their use (Chuda and Adamus 2009) [22]. Interspecific hybridization in genus *Allium* allows researchers to transfer useful traits from closely related *Alliums* into bulb onion, and introgressions from *Allium roylei*, *Allium galanthum* and *Allium fistulosum* have opened new options for improvement (Vu *et al* 2012) [102]. These species can be utilized to develop onion cultivars resistant against various diseases along with desirable agronomic traits, such as earliness and better rooting system.

Table 1: Major *Allium* gene Banks in the World

Name of Institutes	No. of Accessions	Web page	Country
AVRDC – The World Vegetable Center	1082	http://avrdc.org/	Taiwan
Directorate of Onion and Garlic Research	2050	http://www.dogr.res.in/	India
European Cooperative Programme for Plant Genetic Resources	14 4002	http://eurisco.ipk-gatersleben.de	Germany
N.I. Vavilov Research Institute	1888	http://www.vir.nw.ru/	Russia
National Institute of Agrobiological Sciences	1352	http://www.nias.affrc.go.jp/index_e.html	Japan
Royal Botanic Gardens	1100	http://www.kew.org/	UK
US Department of Agriculture	1304	http://www.usda.gov/wps/portal/usda/usdahome	USA
Warwick Crop Center	1755	http://www2.warwick.ac.uk/fac/sci/lifesci/wcc/gru/	UK

(Khosa *et al* 2016) [59]

(2) Need of marker technology: Characterization and grouping based on phenotype are influenced by environmental variations; molecular markers are preferred because of polymorphic nature, co-dominance, high reproducibility and easy exchange of data between laboratories (Joshi *et al* 1999) [54]. There are different marker systems available for crop plants such as RFLP, RAPD, ISSR, SSR, AFLP, SCAR, CAPS and SNP etc (Semagn *et al* 2006) [92] but SSR markers have provided the greatest insights into onion diversity (Khosa *et al* 2014) [60]. Several studies have revealed that short- and long-day germplasm are genetically distinct and these two groups subdivide further based on geographical origins (Mallor *et al* 2014) [71]. Indian short-day germplasm is more diverse than other exotic germplasm and therefore might provide a source of novel alleles for onion breeding (Baldwin *et al* 2012) [8]. Given the genetic diversity of the short-day germplasm from Central Asia and India, it would be also worth screening this material for resistant against various pathogens that could be utilized in breeding programmes.

(3) Diversity analysis and varietal identification of *Alliums:* The knowledge of genetic diversity helps in the efficient management of germplasm and selection of parents for crossing so in these progress 90 RAPD primers grouped 24 onion cultivars into northern and southern regions of India (Sangeeta *et al* 2006) [88], ten varieties of onion *(A. cepa* L.) were analysed, Bermis and India-2 were more dissimilar and Faridpuri and Bhati were the most genetically similar (Maniruzzaman *et al* 2010) [72]. Thirty-two onion germplasm resources analysed using ISSR were divided into five groups; the first group Yellow Sweet Spanish system, the second Bejo Daytona cultivar, the third Yellow Globe system, the fourth Yellow Globe Danvers system and the fifth group of Yellow Danvers system (Qijiang & Jia 2007) [88]. The genetic fidelity of *A. ampeloprasum* L. and *A. sativum in vitro* regenerated clones was studied by Gantait *et al.* (2010) [40] using 10 ISSR while Jakse *et al* (2005) [50] identified 398 SNP, indels and SSRs which distinguished 35 elite onion populations.

(4) Development of SSRs in *Alliums*: With the development of different tools and techniques SSR development has been initiated in *Alliums*. To establish a genetic basis for the breeding of Japanese bunching onion, Tsukazaki *et al* (2008) [99] isolated more than hundreds of SSR clones from size-fractionated genomic DNA libraries. They were highly polymorphic and applicable to the related species such as bulb onion and these can be used for cultivar identification and hybrid purity determination, marker-assisted selection etc. The MISA (MIcroSAtellite identification tool) program revealed 336 dinucleotide to hexa-nucleotide SSRs among 313 unique onion ESTs, representing a frequency of 1 SSR/25 kb similar to 1 SSR/27.2 kb observed in a survey of higher plants (Cardle *et al* 2000) [18]. Baldwin *et al* (2010) [9] reported the application of Roche 454 technology to develop sequence resources for population analyses and genetic mapping. So far 170 genomic SSRs, 9 EST-SSRs, 31 Indels and 156 CAPS markers have been developed.

Table 2: Development of simple sequence repeat (SSR) in *Alliums.*

Method	Marker Developed	References
MISA programme	revealed 336 SSRs, 1 SSR/25kb	Cardle *et al* (2000) [18]
Normalized cDNA library	11 008 unique ESTs generate	Joseph *et al* (2004)
Large scale sequencing of SSR-enriched genomic DNA libraries	1796 SSRs isolated	Tsukazaki *et al* (2007) [99]
Size fractionated genomic library	32 SSRs and 18 bulb onion expressed sequence tags (EST)	Tsukazaki *et al* (2008) [99]
Roche 454 technology	170 genomic SSRs, 9 EST SSRs,156 CAPS markers developed	Baldwin *et al* (2010) [9]

Varietal development in onion

(1) Different coloured varieties: Systematic breeding programme was started as early as 1960 at Pimpalgaon Baswant, Nashik and later at IARI, New Delhi. Early varieties developed through selection procedure. The programme was further strengthened under a coordinated project by institutes of the Indian Council of Agricultural Research at National Horticultural Research and State Agricultural Universities (SAUs). As a result, 45 varieties of onion (including two F1 hybrids and 6 varieties of multiplier onion) have been developed and released.

Table 3: Varieties from Different Organization

S. No.	Organization	Variety	Bulb Colour	Planting Season	Year of Release
1.	MPKV, Rahuri	Phule Suvarna	Yellow	Rabi and late Kharif	2001
		Phule Samarth (S-1)	Red	Late Kharif	2006
2.	IARI, N.Delhi	Selection 126	Brown	Rabi	2012
3.	IIHR, Bangalore	Arka Swadista	White	Rabi	2010
		ArkaVishwas	Dark red	Kharif and Rabi	2011
4.	HAU, Hissar	HOS-1	Red	Rabi	2006
5.	NHRDF, Nasik	NHRDF Red (L-28)	Red	Rabi	2006
		NHRDF Red (L-355)	Red	Rabi	2012
6.	PDKV, Akola	PKV White	White	Rabi	2009
7.	PAU, Ludhiana	Punjab Naroya	Red	Rabi	1995
		Punjab White	White	Rabi	1997
8.	RARS, Durgapura	RO 252	Red	Rabi	2010
9.	DOGR, Rajgurunagar	Bhima Shubhra	White	Kharif and late Kharif	2010
		Bhima Dark Red	Red	Kharif	2012

(3) White onion varieties: Onion varieties for dehydration should be pure white, globose in shape, thin necked, free from greening and moulds, with high pungency, TSS and yield, and, show field tolerance/resistance to diseases and pests. Bajaj *et al* (1979) [7] identified cv. Punjab-48 as most suitable for dehydration on account of its TSS (14.6%). Raina *et al* (1988) [85] recorded maximum (15.8%) TSS in Texas Yellow, followed by Punjab Selection (13.3%), Udaipur-102 (13.5%) and Punjab-48 (13.4%).

Table 4: Performance of White Onion Varieties Developed in India.

Name of the variety	Source	TSS (%)	Average yield (t/ha)
Pusa White Round	IARI, New Delhi	11.13	30.0 – 32.5
Pusa White Flat	IARI, New Delhi	10.00	32.5 – 35.0
Udaipur 102	RAU, Udaipur	10.06	30.0 – 35.0
Agrifound White	NHRDF, Nashik	10.76	20.0 – 25.0
Phule Safed	MPKV, Rahuri	10.13	25.0 – 30.0
PKV White	PDKV, Akola	09.55	25.0 – 30.0
N-257-9-1	Agril. Deptt., M.S.	10.00	25.0 – 30.0
Punjab-48	PAU, Ludhiana	11.00	30.0 – 32.5
V-12	Jain Food Park, Jalgaon	15.00	35.0 – 40.0
Nimar Local	Land Race, M.P.	12.50	25.0 – 30.0
Talaja Local	Land Race, Bhavnagar	12.00	25.0 – 30.0

Production of doubled haploids in onion

Onion populations possess deleterious recessive alleles, and due to inbreeding depression, breeding lines can be selfed for only up to two or three generations. Thus, with conventional breeding it is difficult to obtain homozygous inbreds for complete genetic and phenotypic uniformity in the resultant hybrid. Doubled haploid (DH) production is an alternative strategy for complete homozygosity and phenotypic uniformity to obtain inbred lines in onion (Bohanec 2002) [12]. The major factors affecting haploid induction include genotype, physiological condition of donor plants, developmental stage of the gametes, pre-treatment, composition of culture medium, and physical factors during tissue culture (Murovec and Bohanec 2012) [77]. It has been observed that response of anther to haploid induction is not successful in onion (Keller and Korzum 1996) [57]. A high rate of success in onion through gynogenesis was observed by Bohanec (2009).

(A) Factors affecting haploid induction

(A1) Choice of explants: Gynogenic haploid induction could be achieved through culture of un-pollinated ovules/ ovaries/

whole flower-buds (Bohanec, 2002) [12]. Keller (1990) [58] observed that ovule culture was the most laborious and yielded the lowest number of embryo regenerants. Therefore, this is no longer used for haploid induction in onion (Bohanec, 2002) [12]. Flower bud culture is the simplest way of inducing gynogenic haploids in onion and has been used in many recent studies by various workers.

(A2) Developmental stage: Michalik *et al* (2000) [75] concluded that small young buds of 2.8-3mm length produced significantly fewer embryos than older ones of 3.5-4.5mm length, while displaying genotype specificity.

(A3) Pre-treatment: It's maximizing gynogenic responsiveness in cultured flower-buds of onion across its flowering season. Flower buds excised from stock plants maintained at 15ºC were ten times more responsive than those taken from plants raised under glasshouse conditions, or held at 10ºC.

(A4) Media composition: The three most-often used combinations of macro- and micro-elements have been reported as B5 (Gamborg *et al* 1968) [39], BDS (Dunstan and Short, 1977) [27] and MS (Murashige and Skoog, 1962) [76]. Chen *et al* (2010) [20] mentioned that organic-nitrogen source, carbohydrates and growth regulators are the most-often modified components**.** Growth regulators in different media combinations required for successful gynogenesis are tabulated below:

Table 5

Growth Regulators	Induction Media mg/l	Reference
IBA + BAP	2.03+1.25	Keller (1990) [58]
2,4-D+BAP; NAA+BAP	2+0.12,1+0.12	Campion *et al* (1992) [17]
BA+2,4-D	2+2	Puddephat (1999) [83]
2,4D+BA+Putrescine+Spermidie	0.01mM+0.01mM+2mM+0.1mM	Ebrahimi and Zamani (2009) [35]
2,4-D + BAP	2+2	Jakse *et al* (2010) [51]
Growth Regulators	**Regeneration Media mg/l**	**Reference**
NAA+2,4D+IAA+BAP+2iP	1+0,4+1,5+2+2	Campion and Alloni (1990) [16]
IBA+BAP+GA_3	2+0,1,2+3, 5	Keller 1990 [58], Campion *et al* (1992) [17]
NAA+2iP	1+2	Bohanec *et al* (1995) [13]
2iP+ IAA	2+1.5	Puddephat (1999) [83]

(A5) Effect of genotype

Geoffriau *et al* (1997) [41] reported that among genetic structures, inbreds regenerated significantly better than synthetics. Regenerants from inbreds were the most vigorous, whereas, synthetics were confirmed to be good donor material for quality embryos.

(B) Genome-doubling procedures

Grzebelus *et al* (2004) [45] reported oryzalin, trifluralin and APM as better agents than colchicines for *in vitro* chromosome doubling in onion tissue. However, APM is recommended due to its low toxicity. Alan *et al* (2007) [4] compared the efficiencies of three antimitotic agents (APM, colchicines and oryzalin) and recommended APM (100 or 150µM) due to its low toxicity and ability to yield results comparable to that with colchicine (750 or 1000µM).

It can be concluded that complete homozygosity through DH approach can be attained in less time than traditional breeding approaches.

Genetic transformation in onion

Species hybridization for plant improvement has always been an important tool for the introduction of genetic variation in the breeding of new cultivars, as wild relatives of cultivated species contain gene reservoirs for agronomically useful traits. With respect to sexual hybridization it is known already for a long time that the transfer of genes from one species to another species can be difficult. This proved to be also true for *Allium* (Kik 2002) [63]. However an important breakthrough in this area has recently been accomplished, namely Khrustaleva & Kik (1998, 2000) [61, 62]. They showed that the hybridization barrier between *A. fistulosum* and *A. cepa* can be overcome using *A. roylei* as a bridging species. This results in highly segregating so-called bridge-cross populations [*A. cepa* X (*A. fistulosum* X *A. roylei*)]. Especially the presence of many disease and pest resistances in *A. fistulosum,* but also in *A. roylei,* makes this gene pool approach very attractive for plant breeding purposes.

(1) Regeneration system: The development of an efficient system for genetic transformation is a valuable extension of the gene transfer tools for further crop improvement. In *Allium*, various plant regeneration systems have been developed using different starting material. Eady (1995) [29] reviewed the different source materials used for *in vitro* culture of *Allium* species. The most successful regeneration systems in *Allium* use (im)mature embryos, root tips (segments), flower buds, suspension cultures or protoplasts as starting materials.

(2) Transformation system: Recently, reports were published showing that genetic transformation has become possible in *Allium* and this without any doubt is a major step forward (Eady 2002) [30]. With report to particle bombardment, Klein *et al* (1987) [69] developed the first one a high-velocity microprojectile method and demonstrated that epidermal tissue of onion could take up foreign DNA sequences. Eady *et al* (2000) [34] developed a stable transformation protocol using immature embryos of *A. cepa* via *Agrobacterium tumefaciens*. Due to the aforementioned efforts in developing reliable transformation systems for *Allium* crops nowadays transgenic shallot and garlic plants containing *Bt* resistance genes have been produced which confer resistance to beet armyworm (*Spodoptera exigua*) (Zheng *et al* 2004a, 2004b) [104, 105]. Furthermore, transgenic plants containing herbicide resistance and antisense versions of *alliinase* genes are available (Eady 2002) [30].

Table 6: Genetic transformation in onion by different methods.

Species	Target tissue	Method	Result	References
A. cepa	Bulb	*A. tumefaciens*, *A. rhizogenes*	Tumorigenic response and opine production	Dommisse *et al* (1990)
A. cepa	Microbulbs from germinating seeds	Particle bombardment, *A. tumefaciens*	Transient expression of *gusA*	Eady *et al* (1996) [31]
A. cepa	Immature embryo	*A. tumefaciens*	Stable expression of *nptII* and *m-gfp5-ER*	Eady *et al* (2000) [34]
A. cepa	Calli derived from mature embryo	*A. tumefaciens*	Stable expression of *gusA* and *hpt*	Zheng *et al* (2001) [106]
A. cepa	Immature embryo	*A. tumefaciens*	Stable expression of *bar* and antisense versions of alliinase genes	Eady *et al* (2002) [30], (2003) [28]
A. cepa	Calli derived from mature embryo	*A. tumefaciens*	Stable expression of *gusA, hpt* and *Bt* genes (*cry1Ca* or *H04*)	Zheng *et al* (2004)

Male sterility in onion

In onion, male sterility is due to interaction of cytoplasm and nuclear gene, *i.e.* cytoplasmic genetic male sterility (CGMS). The first male sterile plant was reported within the progenies of an onion cultivar 'Italian Red' (Jones and Emsweller, 1936) [53], which was cytoplasmically inherited, and male sterility was under the control of a single recessive nuclear restorer locus (Jones and Clarke, 1943) [52]. Male sterile plants occur in natural populations, but the proportion of male sterile plants varies with the site and season. Berninger (1965) [10] identified a new sterile cytoplasm (T cytoplasm) in French cultivar 'Jaune Paille des Vertus'. Fertility restoration in T-cytoplasm is controlled by three independent genes (Scheweisguth, 1973) [89]. Globally, the CGMS system has been commercially exploited in onion for hybrid development over the last four decades. The first F_1 hybrid, VL- 67, was released in 1973, and thereafter, an improved F_1 cross "BYG-2207 x Almora Selection-2", was identified at VPKAS, Almora in 1976 and these hybrids were developed using exotic CGMS lines. This male sterility has been transferred to several breeding lines by backcross breeding method. Transfer of cytoplasm from related species into cultivated populations may produce new sources of CGMS (Havey 1999) [49]. In an attempt to diversify the cytoplasm conditioning male sterility, the cytoplasm of *Allium galanthum* was backcrossed for seven generations to bulb-onion populations. The flowers of galanthum cytoplasmic populations possess upwardly curved perianth and filaments with no anthers, making identification of male sterile plants easier than for either S- or T cytoplasmic male sterile onion plants. It is understood that these *galanthum*-cytoplasmic onion populations can be used as alternative male-sterile cytoplasm for the diversification of hybrid onion seed production. Worldwide, more than 50% of currently cultivated onion varieties are F_1 hybrids derived from S-cytoplasm (Pelletier *et al* 1995) [80]. Development of onion hybrids faces major obstacles because of a high rate of inbreeding depression while developing inbreds.

(1) Marker for cytoplasmic male sterility (CMS) in onion: A low-density genetic map of onion (*A. cepa* L.) was developed, comprised of RFLPs and distinguished normal fertile (N) and sterile (S) cytoplasm of onion. There was a correlation between expected and observed numbers of plants maintaining CMS (Havey *et al* 2001) [48]. At least three restorer genes are involved in the restoration of fertility in CMS-T male-sterile while fertility restoration in CMS-S is controlled by a single gene only, rendering it suitable for the establishment of molecular breeding systems (Kim *et al* 2009) [66]. One SCAR marker and one RAPD marker were identified, which could distinguish between N and S cytoplasm in several Welsh onion cultivars, confirmed by Southern blotting (Gai & Meng 2010) [38].

Table 7: Molecular Markers for Cytoplasmic Male Sterility (CMS) analysis in *Alliums.*

Marker	Application	Refernces
RFLP	identify the cytoplasmic genotypes	Szklarczyk *et al* (2002) [96]
PCR-RFLP	distinguish male-fertile (N) and male-sterile (S) cytoplasm	Cho *et al* (2006) [21]
RFLP	CMS-T and CMS-S cytoplasm type identification	Kim *et al* (2009) [66]
RAPD, SSR, RFLP	genomic and mitochondrial genome diversity	Chaurasia *et al* (2010) [19]
RAPD and CAPS	high-resolution linkage map of the Ms locus	Park *et al* (2013) [79]
Chloroplast and mitochondrial markers	study of mitochondrial genome rearrangements	Kim *et al* (2013) [64]

Quality improvement in onion

The quality of onion cultivars is determined by bulb colour (anthocyanin and flavonoid content), firmness, number of scales, number of growing points, neck thickness, total soluble solids (TSS), pungency and antioxidants (Brewster 2008, Goldman 2011) [14, 43]. Onion-processing industries require cultivars with single-centre bulbs, high TSS and dry matter, traits that are also important for storage (McCallum 2007) [73]. Red onions tend to have higher antioxidant activities than yellow and white onions, although it is possible to breed yellow onions with high antioxidant activities due to their high total phenolic contents. There is huge demand for single centred (one growing point) bulbs for fresh market and onion ring industries.

(1) Molecular Markers for quality traits of onion: The flavour precursors of onion are 1-propenyl, propyl and methyl cysteine sulphoxides (Randle & Lancaster 2002) [87]. McCallum *et al* (2006) [72] found a polymorphic SSR marker which exhibited strong disequilibrium with bulb fructan content and was mapped to chromosome 8 in the interspecific population *A. cepa* × *A. roylei*. QTL analysis revealed significant associations of both pungency and bulb soluble solids content with marker intervals on chromosomes 3 and 5 (McCallum *et al* 2007) [73]. The non-structural dry matter content of onion bulbs consists principally of fructose, glucose, sucrose and fructans. Raines *et al* (2007) [86] constructed a cDNA subtraction library to differentiate the high and low fructan accumulating background.

(2) Colour improvement in onion: Bulb colour is one of the important traits in onion (*A. cepa* L). The bulb colour is due to flavonoid compounds and 54 kinds of flavonoids have been reported in onion (Slimestad *et al* 2007) [93]. Unusual gold-coloured onions showed a reduced amount of quercetin, the most abundant flavonoid in onions. Kim *et al* (2004) [64] identified critical mutations in the *chalcone isomerase* (CHI) gene causing gold onions. The colour difference between yellow and red onions was revealed suggesting the involvement of two complementary genes in anthocyanin production in the F1 hybrids (Kim *et al* 2005) [67].

Sources of resistance to onion diseases and pests

Onions are prone to a wide range of insect pests and diseases. Among them, downy mildew, fusarium basal rot (*Fusarium oxysporum*), pink root (*Pyrenochaeta terrestris*), white rot (*Sclerotium cepivorum*), black mould (*Aspergillus niger*), purple blotch (*Alternaria porri*), stemphylium blight (*Stemphylium vesicarium*), iris yellow spot virus (IYSV) and onion yellow dwarf virus (OYDV) are the major diseases affecting bulb and seed crops of onion (Brewster 2008) [14]. Seven species of Allium, *viz.*, *Allium fistulosum, A. roylei, A. bouddhae, A. schoenoprasum, A. galanthum, A pskemense, A. oschaninii* have shown varying degree of resistance from immune to susceptible to these diseases. Of these, *A. fistulosum* and *A. roylei* have shown multiple resistances to Botrytis leaf blight, downy mildew, anthracnose, Fusarium basal rot etc. *A. bouddhae, A. schoenoprasum* showed immune reaction to onion blast (Schwartz and Mohan, 1995) [91]. The introduction of the downy mildew resistance gene (*Pd1* and *Pd2*) from *A. roylei* into edible *Alliums* in recent times using molecular techniques and further using molecular markers to follow through on the assessment of its incorporation is a good example of marker assisted breeding (Brewster 2008) [14]. Resistance to fusarium basal rot has been identified in different germplasms of bulb onion and other alliums and is governed by one, two or multiple genes depending on the germplasm (Taylor *et al* 2013) [97]. Tolerance to white rot, pink root, stemphylium blight, black mould, purple blotch and IYSV has been identified, but complete resistance has not been found in onion or other alliums (Bag *et al* 2014, Kale and Ajjappalavara 2014) [6, 55].

Purple Blotch Disease

Purple blotch, caused by *Alternaria porri* is the most destructive foliar disease, prevalent in all *Allium* growing countries of the world (Kareem *et al* 2012) [56]. It is responsible for causing severe yield losses ranging from 2.5% to 97% in both the bulb and seed crop (Lakra 1999) [70]. Purple blotch control often involves frequent application of fungicides (Priya *et al* 2015) [82], biological control by inoculation of antagonistic fungi and bacteria and genetic engineering towards purple blotch resistance in onion (Eady *et al* 2003) [32]. However, these are mostly time consuming, costly and often ineffective due to the emergence of resistant races of the pathogen. In these circumstances, host resistance breeding could be the most effective way to control purple blotch disease. However, there is only a limited source of naturally available host plants that exhibit resistance against purple blotch. A few onion lines have been identified that exhibit resistance or moderate resistance to purple blotch in field screening conditions (Tripathy *et al* 2013) [98]. Varieties like Punjab-48 (S-48), Punjab Red Round, Punjab Selection and Punjab Naroya (PBR- 5) from PAU; Arka Lalima, Arka Kirthiman and Arka Pitamber from IIHR have been identified that shown resistance to purple blotch.

Among insects, onion thrips (*Thrips tabaci* L.) causes major damage by reducing photosynthetic activity, making an entry point for pathogens and as a vector for IYSV (Bag *et al* 2014) [6]. Germplasm from different countries has been evaluated and a few thrips tolerant sources identified, which can be utilized in breeding programmes (Diaz-Montano *et al* 2011) [25]. Glossy and semi-glossy foliage has been shown to be non-preference to thrips that minimize feeding damage in onion (Damon and Havey 2014) [24]. Genetic studies indicate low heritability for thrips resistance, and it would be desirable to use family based selection to increase genetic gain (Hamilton *et al* 1999) [47]. Several cultivars/germplasm accessions of *Allium* species have been evaluated worldwide and found to possess varying levels of resistance to different biotic stresses.

Table 8: Onion Cultivars Resistance against Pests and Diseases.

Pest	Source of Resistance	References
Fusarium basal rot	Arka Lalima, Arka Kirthiman and Arka Pitamber from IIHR	Somkuwar *et al* (1996) [95]
White rot	IIHR Yellow; Hybrid-3-MR	Somkuwar *et al* (1997) [94]
Purple blotch *(Alternaria porri)*	Fiesta-R, Punjab -48 (S-48), Punjab Red Round, Punjab Selection and Punjab Naroya (PBR- 5) from PAU; Arka Lalima, Arka Kirthiman and Arka Pitamber from IIHR	Potandon Produce L.L.C. (2003) [81] NORV database Gupta and Pathak (1988) [46]
Xanthomonas leaf blight	Cometa, Blanco Duro and Redwing (white and red varieties)-T	Schwartz and Gent (2005) [90]
Thrips	Punjab -48 (S-48), and Punjab Selection from PAU; Arka Lalima, Arka Kirthiman and Arka Pitamber from IIHR	Alimousavi *et al* (2007) [5]
Pink root disease	Texas Early Grano, Yellow Sweet Spanish (commercially exotic resistant cultivars)	Nasr Esfahani and Ansari Pour, (2008) [78]
Stemphylium leaf blight	PI 208733 ; PI 280565 ; PI 461393 ;Asagikei- Kujyo ; PI 461398; Kujyo-futo; PI461402; Shiobara-bansei	USDA, ARS, National Genetic Resources Program. [Online Database] (2009) [100]

Storage of onion

(1) Application of pre-harvest and spray chemicals for onion storage

Bufler (2009) [15] found that Copra onion variety held in continuous ethylene (10.6 Ml L-1) had reduced sprout growth compared with those held in air. Adamicki, (2004) [2] reported that the application of ethephon to onion plants 2 weeks prior to harvest was found to reduce sprout incidence by 5% after 32 weeks of storage at 0°C; however, no significant reduction in rooting was observed. Cools *et al*. (2011) [23] reported that ethylene can suppress sprouting while the ethylene-binding inhibitor 1-methylcyclopropene (1-MCP) can also suppress sprout growth Bulbs fumigated with sulphur dust before storage under natural ventilation were found to have better shelf life for 16 weeks (Abbey, 2000) [1].

(2) Storage structures

(2a.) Controlled atmospheric storage: Yamashita *et al.* (2009) [103] tested 1 °C and 80% RH in 1% O_2 + 1% CO_2 for storage up to 196 days and found that sprouting and root growth were inhibited in CA storage and 98.2% were

considered marketable, compared with 69.2% for those stored at -0.5 °C and 80% RH in air.

(2b.) Modified atmospheric storage

Adamicki *et al.* (1977) [3] found that internal breakdown was observed in 68.6% of the bulbs when they were stored at 1 °C in sealed PE bags with a CO_2 concentration higher than 10%; they also found a similar disorder in bulbs stored at 5 °C in sealed PE bags, and this was probably due to the very high concentration of CO_2 of 28.6%.

To reduce losses during storage modified or controlled atmospheric storage with proper temperature management, shall control sprouting and weight loss against the pre-harvest chemical spray and prolong the time of onion storage.

Future strategies

- Shifting toward globalization and industrialization
- Development of high yielding and disease resistant F_1 hybrids using CMS lines
- Identification of responsive gynogenic material for DH production
- Transfer of disease and pest resistant genes from other species of *Allium* to cultivated *Allium cepa*
- Development of biotic and abiotic resistant inbred lines from local variable genetic material for production of F_1 hybrids
- More emphasis on quality retention in onion breeding
- Better availability of suitable F_1 hybrid seed

So in conclusion, further efforts should be made towards different approaches like development of high yielding and disease resistant F_1 hybrids using CMS lines and identification of responsive gynogenic material for double haploid production. Emphasis should also be made towards transfer of disease and pest resistant genes from wild species of *Allium* to cultivated *Allium cepa,* development of biotic and abiotic resistant inbred lines, quality retention and better availability of suitable F_1 hybrid seeds.

References

1. Abbey I. Effect of poultry manure and post production application of fungicide on the shelf life of onion *cv.* Bawku Red. Crop Research. 2000; 20(1):87-92.
2. Adamicki F. Effects of pre-harvest treatments and storage conditions on quality and shelf-life of onions. *Acta Hortic.* 2004; 688:26-33.
3. Adamicki F, Dyki B, Malewski W. Effects of carbon dioxide on the physiological disorders observed in onion bulbs during CA storage. *Qualitas Plantarum*. 1977; 27:239-48.
4. Alan AR, Lim W, Mutschler MA, Earle ED. Complementary strategies for ploidy manipulations in gynogenic onion (*Allium cepa* L.). *Pl. Sci.*, 2007; 173:25-31.
5. Alimousavi SA, Hassandokht MR, Moharramipour S. Evaluation of Iranian Onion Germplasms for Resistance to Thrips. International Journal of Agriculture and Biology. 1560- 8530. 2007; 9:897-900.
6. Bag S, Schwartz HF, Cramer CS, Havey MJ, Pappu HR. Iris yellow spot virus (Tospovirus: *Bunyaviridae*) from obscurity to research priority. *Mol. Plant Pathol.* 2014; 16: 224-37.
7. Bajaj KL, Kaur G, Singh T. Lachrymatory factor and other chemical constituents of some varieties of onion. J. Plant Food. 1979; 3:119-203.
8. Baldwin S, Joyce MP, Wright K, Chen L, McCallum J. Development of robust genomic simple sequence repeat markers for estimation of genetic diversity within and among bulb onion (*Allium cepa* L.) populations. Mol. Breed. 2012; 30:1401-11.
9. Baldwin SJ, Meeghan PJ, Roopashree R, Mark F, Ross C, Richard M *et al*. Application of 454™ sequencing for marker development in onion. In: 18th Conf. Plant and Animal Genomes. San Diego, 2010.
10. Berninger E. *Contribution a letude de la sterilite male de loifnon (Allium cepa).* Annals Amelior Plant. 1965; 15:183-99.
11. Bohancec B. Doubled Haploids via Gynogenesis. In: Advances in Haploid Production in Higher Plants. Touraev, A., Forster, B.P. and Jain, S.M. (eds.). Springer-Science and Business Media B.V., The Netherlands, 2009, 35-46.
12. Bohanec B. Doubled haploid onions. In: *Allium* crop science: Recent advances. CABI Publishing House, Wallingford, UK, 2002, 145-148.
13. Bohanec B, Jakse M, Ihan A, Javornik B. Studies of gynogenesis in onion (*Allium cepa* L.): induction procedures and genetic analysis of regenerants. Pl. Sci. 1995; 104:215-224.
14. Brewster JL. Onions and other vegetable *Alliums*. CABI Publishers. 2008, 432.
15. Bufler G. Exogenous ethylene inhibits sprout growth in onion bulbs. *Ann Bot* (Lond). 2009; 103:23-28.
16. Campion B, Alloni C. Induction of haploid plants in onion (*Allium cepa* L.) by *in vitro* culture of unpollinated ovules. Pl. Cell Tiss. Org. Cult. 1990; 20:1-6.
17. Campion B, Azzimonti MT, Vicini E, Schiayi M, Falavigna A. Advances in haploid induction in onion (*Allium cepa* L.) through *in vitro* gynogenesis. Pl. Sci. 1992; 86:97-104.
18. Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R. Computational and experimental characterization of physically clustered simple sequence repeats in plants. Genetics, 2000; 156:847-54.
19. Chaurasia AK, Adsul GG, Nair D, Subramaniam VR, Krishna B, Sane PV. Diversity in Indian and some exotic onion cultivars as revealed by genomic and mitochondrial DNA. *Acta Horticulturae* (ISHS) 2010; 859:207-20.
20. Chen JF, Cui L, Malik AA, Mbira KG. *In vitro* haploid and dihaploid production *via* unfertilized ovule culture. Pl.t Cell Tiss. Org. Cult. 2010; 68:51-59.
21. Cho KS, Yang TJ, Hong SY, Kwon YS, Woo JG, Park HG. Determination of cytoplasmic male sterile factors in onion plants (*A. cepa* L.) using PCR-RFLP and SNP markers. Molecular Cells. 2006; 21:411-17.
22. Chuda A, Adamus A. Aspects of interspecific hybridization within edible Alliaceae. Acta Physiol. Plant 2009; 31:223-27.
23. Cools K, Gemma A, Chope GA, John PH, Andrew JT, Leon AT. Plant Physiology. 2011; 156:1639-52.
24. Damon S, Havey MJ. Quantitative trait loci controlling amounts and types of epicuticular waxes in onion. J. Am. Soc. Hortic. Sci. 2014; 139:597-602.
25. Diaz-Montano J, Onatno J, Fuchs M, Nault BA, Fail J, Shelton AM. Onion thrips: a global pest of increasing concern in onion. J. Econ. Entomol. 2011; 104:1-13.
26. Dommisse EM, Leung DWM, Shaw ML, Conner AJ. Onion is a monocotyledonous host for Agrobacterium. Plant Science. 1990; 69:249-57.

27. Dunstan DI, Short KC. Improved growth of tissue cultures of onion (*Allium cepa*). Physiol. Plant. 1977; 41:70-72.
28. Eady C, Davis S, Farrant J, Reader J, Kenel F. *Agrobacterium tumefaciens*-mediated transformation and regeneration of herbicide resistant onion (*Allium cepa* L.) plants. Ann. Appl. Biol. 2003; 142:213-17.
29. Eady CC. Towards the transformation of onions (*Allium cepa*). New Zealand Journal of Crop and Horticultural Science. 1995; 23:239-50.
30. Eady CC. Genetic transformation of onions. In: H. D. Rabinowitch & L. Currah (Eds.), *Allium* Crop Science: Recent Advances, CAB International, Wallingford, UK, 2002, 119-144.
31. Eady CC, Butler RC, Suo Y. Somatic embryogenesis and plant regeneration from immature embryo cultures of onion (*Allium cepa* L.). Plant Cell Reports. 1996; 18:111-16.
32. Eady CC, Davis S, Farrant J, Reader J, Kenel F. *Agrobacterium tumefaciens*-mediated transformation and regeneration of herbicide resistant onion (*Allium cepa*) plants. Ann. Appl. Biol. 2003; 142:213-17.
33. Eady CC, Kamoi T, Kato M, Porter NG, Davis S, Shaw M *et al*. Silencing onion lachrymatory factor synthase causes a significant change in the sulfur secondary metabolite profile. Plant. Physiol. 2008; 147:2096-2106.
34. Eady CC, Weld RJ, Lister CE. *Agrobacterium tumefaciens*-mediated transformation and transgenic-plant regeneration of onion (*Allium cepa* L.). Plant Cell Reports, 2000; 19:376-81.
35. Ebrahimi R, Zamani Z. Effect of polyamines on *in vitro* gynogenesis of onion (*Allium cepa* L.). Am- Eurasian J. Sustain. Agri. 2009; 3:71-74.
36. Faostat. Onwards Onion production of top five producers, 2014. URL http://faostat3.fao.org/browse/Q/QC/E.
37. Fritsch RM, Friesen N. Evolution, domestication and taxonomy. In: H. D. Rabinowitch, and L. Currah (eds), *Allium* Crop Science: *Recent Advances*, 5-30. CAB International, Wallingford, UK, 2002.
38. Gai S, Meng X. Application of molecular markers linking to cytoplasmic male sterile loci to assist maintainer line selection and their selection efficiency in Welsh onion (*A. fistulosum* L.). Agricultural Sciences in China. 2010; 9:1571-76.
39. Gamborg OC, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exptl. Cell Res. 1968; 50:151-58.
40. Gantait S, Mandal N, Bhattacharyya S, Das PK. Determination of genetic integrity in long-term micro-propagated plantlets of *A. ampeloprasum* L. using ISSR markers. Biotechnology, 2010; 9:218-23.
41. Geoffriau E, Kahane R, Rancillac M. Variation of gynogenesis ability in onion (*Allium cepa* L.) Euphytica, 1997; 94:37-44.
42. Glaszmann JC, Kilian B, Upadhyaya HD, Varshney RK. Accessing genetic diversity for crop improvement. Curr. Opin. Plant. Biol. 2010; 13:1-7.
43. Goldman IL. Molecular breeding of healthy vegetables. EMBO Rep. 2011; 12:96-102.
44. Gorinstein S, Drzewiecki J, Leontowicz H, Leontowicz M, Najman K, Jastrzebski Z. Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish, Ukrainian, and Israeli garlic. J. Agric. Food. Chem. 2005, 2726-32.
45. Grzebelus E, Adamus A. Effect of anti-mitotic agents on development and genome doubling of gynogenic onion (*Allium cepa* L.) embryos. Pl. Sci. 2004; 167:569-74.
46. Gupta RBL, Pathak VN. Survival of *Alternaria porri*, inducer of purple blotch of onion. Indian Phytopathology. 1988; 41:406-09.
47. Hamilton BK, Pike LM, Sparks AN, Bender DA, Jones RW, Candeia J *et al.* Heritability of thrips resistance in the 'IPA-3' onion cultivar in South Texas. *Euphytica,* 1999; 109: 117-22.
48. Havey M, King JJ, Bradeen JM, Bark O, Sato Y, Gökçe AF. A low-density genetic map of onion (*A. cepa* L.) and its use for marker-facilitated selection of maintainer lines. *Acta Horticulturae* (ISHS), 2001; 555:87-89.
49. Havey MJ. Seed yield, floral morphology and lack of male fertility restoration of male sterile onion *(Allium* cepa*)* populations possessing the cytoplasm of *Allium galanthum*. J. Amer. Soc. Hort. Sci. 1999; 124:626-29.
50. Jakse J, Martin W, Mccallum J, Havey JM. Single nucleotide polymorphism, Indels, simple sequence repeats for onion cultivar identification. Journal of Amer-ican Society for Horticultural Science. 2005; 130:912-17.
51. Jakse M, Hirschegger P, Bohanec B, Havey MJ. Evaluation of gynogenic responsiveness and pollen viability of selfed doubled haploid onion lines and chromosome doubling *via* somatic regeneration. J. Amer. Soc. Hortl. Sci. 2010; 135:67-73.
52. Jones HA, Clarke AE. Inheritance of male sterility in onion and the production of hybrid seed. Proc. Amer. Soc. Hort. Sci. 1943; 43:189-194.
53. Jones HA, Emsweller SL. A male sterile onion. Proc. Amer. Soc. Hort. Sci. 1936; 34:582-85.
54. Joshi SP, Ranjekar PK, Vidya SG. Molecular markers in plant genome analysis. Current Science. 1999; 77:230-40.
55. Kale SM, Ajjappalavara PS. Evaluation of onion genotypes against purple blotch (*Alternaria porri*). Asian J. Hortic. 2014; 9:274-75.
56. Kareem MA, Murthy KVMK, Nadaf HA, Waseem MA. Effect of temperature, relative humidity and light on lesion length due to *Alternaria porri* in onion. Asian J. Environ. Sci. 2012; 7:47-49.
57. Keller ERJ, Korzum L. Haploidy in onion (*Allium cepa* L.) and other *Allium* species. In: *In vitro* haploid production in higher plants.Vol 3. Jain, S.M., Sopory, S.K. and Veilleux, R.E. (eds.). Springer Science and Business Media, B.V. Netherlands, 1996, 51-75.
58. Keller J. Culture of unpollinated ovules, ovaries and flower buds in some species of the genus *Allium* and haploid induction via gynogenesis in onion (*Allium cepa* L.). *Euphytica.* 1990; 47:241-247.
59. Khosa JS, McCallum J, Dhatt AS, Macknight RC. Enhancing onion breeding using molecular tools. Plant Breeding. 2016; 135:9-20.
60. Khosa JS, Negi KS, Dhatt AS, Khar A. Utility of Simple Sequence Repeat (SSR) markers to realize worth of germplasm in genus *Allium*. Indian J. Plant Genet. Resour, 2014; 27:238-45.
61. Khrustaleva LI, Kik C. Cytogenetical studies in the bridge cross *Allium cepa* x (*A. fistulosum x A. roylei*). *Theor. Appl. Genet*. 1998; 96:8-14.
62. Khrustaleva LI, Kik C. Introgression of *Allium fistulosum* into *A. cepa* mediated by A. roylei Theor. Appl. Genet. 2000; 100:17-26.
63. Kik C. Expoitation of wild relatives for the breeding of *cultivated Allium* species. In: H. D. Rabinowitch & J. L.

Brewster (Eds.), *Allium* Crop Science: Recent advances, CAB International, Wallingford, UK, 2002, 81-100.
64. Kim S, Bang H, Patil BS. Origin of three characteristic onion (*Allium cepa* L.) mitochondrial genome rearrangements in *Allium* species. *Scientia Horticulturae*. 2013; 157:24-31.
65. Kim S, Jones R, Yoo K, Pike LM. Gold colour in onions (*A. cepa*): a natural mutation of the *chalcone isomerase* generesulting in a pre-mature termination codon. Molecular Genetics and Genomics. 2004; 272:411-19.
66. Kim S, Lee ET, Kim CW, Yoon MK. Distribution of three cytoplasm types in onion (*A. cepa* L.) cultivars bred in Korea and Japan. Korean Journal of Horticultural Science & Technology. 2009; 27:275-79.
67. Kim S, Rick J, Kil-Sun Y, Leonard MP. The *L* locus, one of complementary genes required for anthocyanin production in onions (*A. cepa* L.), encodes anthocyanidin synthase. Theoretical and Applied Genetics. 2005; 111:120-27.
68. King JJ, Bradeen JM, Bark O, McCallum J, Havey MJ. A low density genetic map of onion reveals a role for tandem duplication in the evolution of extremely large diploid genome. Theor. Appl. Genet. 1998; 96:52-62.
69. Klein TM, Wolf ED, Wu R, Sanford JC. High-velocity microprojectiles for delivering nucleic acids into living cells. Nature. 1987; 327:70-73.
70. Lakra BS. Development of purple blotch incited by *Alternaria porri* and its losses in seed crop of onion (*Allium cepa*). Indian J. Agric. Sci. 1999; 69:144-46.
71. Mallor C, Arnedo-Andres MS, Garces-Claver A. Assessing the genetic diversity of Spanish *Allium cepa* landraces for onion breeding using microsatellite markers. Sci. Hortic. 2014; 170:24-31.
72. Maniruzzaman ME, Haque MM, Haque MA. Molecular characterization of onion (*A. cepa* L.) using RAPD markers. Bangladesh Journal of Agricultural Research. 2010; 35:313-22.
73. McCallum J. Onion. In:C. Kole (ed.), Genome Mapping and Molecular Breeding in Plants Springer. 2007; 5:331-42.
74. McCallum J, Andrew C, Meeghan PJ, Martin S, Ruth B, John S *et al*. Genetic mapping of a major gene affecting onion bulb fructan content. Theoretical and Applied Genetics. 2006; 112:958-96.
75. Michalik B, Adamus A, Nowak E. Gynogenesis in Polish onion cultivars. J. Pl. Physiol. 2000; 156:211- 16.
76. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962; 15:473-97.
77. Murovec J, Bohanec B. Haploids and Doubled Haploids in Plant Breeding. In: Plant Breeding. Abdurakhmonov, I. (ed.). InTech, Europe, 2012, 87-106.
78. Nasr EM, Ansari PB. Differences in resistance in onion cultivars to pink root disease in Iran. Journal of General Plant Pathology. 2008; 74:46-52.
79. Park J, Cho DY, Moon JS, Yoon MK, Kim S. Development of functional markers for detection of inactive *DFR-A* alleles responsible for failure of anthocyanin production in onions (*Allium cepa* L.). Korean Journal of Horticultural Science & Technology. 2013; 31:72-79.
80. Pelletier G, Ferault M, Lancelin D, Boulidard L, Dore C, Bonhomme S *et al*. Engineering of cytoplasmic male sterility in vegetables by protoplast fusion. *Acta Horticulturae*, 1995; 392:11-17.
81. Potandon Produce LLC, 2003. http://www.potandon.com/ss_onion_diseases.htm
82. Priya RU, Sataraddi A, Darshan S. Efficacy of non-systemic and systemic fungicides against purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* (Ellis) Cif. Int. J. Recent Scientific Res. 2015; 6:6519-21.
83. Puddephat IJ, Robinson HT, Smith BM, Lynn J. Influence of stock plant pre-treatment on gynogenic embryo induction from flower buds of onion. Pl. Cell Tiss. Org. Cult. 1999; 57:145-48.
84. Qijiang CC, Jia T. Genetic diversity analysis of onion (*A. cepa* L.) germplasm by ISSR markers. Chinese Agricultural Science Bulletin. 2007; 23:126-30.
85. Raina BL, Teotia MS, Pruthi JS, Kalra CL. Studies on the evaluation of some new onion cultivars for their suitability for dehydration. Ind. Food Packer. 1988; 42:7-14.
86. Raines S, Havey MJ, Henson CA. Candidate genes affecting fructan accumulation in onion. Plant and Animal Genome Conference, 2007. Available at http://www.intl-pag.org/15/abstracts/PAG15_P05a_223.html.
87. Randle WM, Lancaster JE. Sulphur compounds in *Alliums* in relation to Flavour quality. In: Rabino-witch H., Currah L. (eds): *Allium* crop science - Recent advances. CABI Publishing, Oxford, 2002, 1-62.
88. Sangeeta KM, Gowda RV, Lalitha A. Analysis of genetic diversity among Indian short-day onion (*A. cepa* L.) cultivars using RAPD markers. Journal of Horticultural Science and Biotechnology. 2006; 81:774-77.
89. Scheweisguth B. Study of a new type of male sterility in onion. Genetics. 1973; 29:569-72.
90. Schwartz H, Gent DH. Xanthomonas leaf blight of onion. no. 2.951, 2005. http://www.ext.colostate.edu/PUBS/Garden/ 02951.html
91. Schwartz HF, Mohan SK. Compendium of onion and garlic diseases. The American Phytopathological Society. St Paul, Minnesota, USA, 1995, 34.
92. Semagn K, Bjørnstad A, Ndjiondjop MN. An overview of molecular marker methods for plants. African Journal of Biotechnology. 2006; 5:2540-68.
93. Slimestad R, Fossen T, Vågen IM. Onions: a source of unique dietary flavonoids. Journal of Agricultural Food Chemistry. 2007; 55:10067-80.
94. Somkuwar RG, Gowda RV, Singh TH. Varietal response to white rot in onion. Madras Agricultural Journal. 1997; 84:343-44.
95. Somkuwar RG, Gowda RV, Singh TH, Pathak CS. Screening of onion for resistance to onion basal rot. Madras Agricultural Journal. 1996; 83:273-275.
96. Szklarczyk M, Simlat M, Jagosz B, Ba G. The use of cytoplasmic markers in onion hybrid breeding. Cell Molecular Biology Letters. 2002; 7:625-34.
97. Taylor A, Vagany V, Barbara DJ, Thomas B, Pink DAC, Jones JE *et al*. Identification of differential resistance to six *Fusarium oxysporum* f sp. *cepae* isolates in commercial onion cultivars through the development of a rapid seedling assay. Plant. Pathol. 2013; 62:103-11.
98. Tripathy P, Priyadarshini A, Das SK, Sahoo BB, Dash DK. Evaluation of onion (*Allium cepa* L.) genotypes for tolerance to thrips (*Thrips tabaci* L.) and purple blotch (*Alternaria porri* (E) Ciferri). Int. J. Biores. Stress Manage. 2013; 4:561-64.
99. Tsukazaki H, Nunome T, Fukuoka H, Kanamori H, Kono I, Ohara T *et al*. Applications of DNA marker technology

in Japanese bunching onion breeding. *Acta Horticulturae* (ISHS), 2008; 770:153-58.
100. USDA ARS, National Genetic Resources Program. Germplasm Resources Information Network-(GRIN) 2009. [Online Database] http://www.ars-grin.gov/cgi-bin/npgs/html/close.
101. Vidyavati HG, Manjunatha H, Hemavathy J, Srinivasan K. Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats. J. Food. Sci. Technol. 2010; 47(1):55-60
102. Vu HQ, Yoshimatsu Y, Khrustaleva LI, Yamauchi N, Shigyo M. Alien genes introgression and development of alien monosomic addition lines from a threatened species, *Allium roylei* Stearn, to *Allium cepa L*. *Theor.* Appl. Genet. 2012; 124:1241-57.
103. Yamashita K, Wataru S, Kazuo F, Hiroshi H. Effects of controlled atmosphere storage on storage life and shelf-life of the Japanese onion cultivar Super-Kitamomiji. 10th International Controlled & Modified Atmosphere Research Conference. Turkey [poster abstract], 2009, 53.
104. Zheng SJ, Henken B, Ahn YK, Krens FA, Kik C. The development of a reproducible *Agrobacterium tumefaciens* transformation system for garlic (*Allium sativum* L.) and the production of transgenic garlic resistant to beet armyworm (*Spodoptera exigua* Hübner). *Molecular Breeding* (In press), 2004a.
105. Zheng SJ, Henken B, de Maagd RA, Purwito A, Krens FA, Kik C. Two different *Bacillus thuringiensis* toxin genes confer resistance to beet armyworm (*Spodoptera exigua* Hübner) in transgenic *Bt*-shallots (*Allium cepa* L.). *Plant Biotechnology Journal* (Submitted), 2004b.
106. Zheng SJ, Khrustaleva L, Henken B, Sofiari E, Jacobsen E, Kik C *et al*. *Agrobacterium tumefaciens*-mediated transformation of *Allium cepa* L.: the production of transgenic onions and shallots. Molecular breeding. 2001; 7(2):101-115.