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Omics and Biotechnology: tools to improve Onion crop production and quality in developing countries

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ABSTRACT

Onion is the second most cultivated vegetable crop and a dietary source of vitamins and flavonoids. Historically, the specie has undergone biotechnological manipulations for crop improvements due to its high amenability to cell and tissue culture. This review begins with a historical viewpoint on onion improvement using biotechnology encompassing ploidy manipulation and applications of cell and tissue culture. The past developments and new approaches for gene transfer to onion are described in this review. Genetic transformation is highly effective for adding single genes to existing elite onion clones with no, or minimal disruptions to their genetic background and represents the only effective way to produce isogenic lines of specific cultivars. This is nearly impossible via traditional breeding due to the high heterozygosity in the diploid onion genome, the genetic integrity of onion clones is lost upon sexual reproduction as a result of viable gene segregation. These genetic attributes have also provided challenges for the development of genetic maps and applications of molecular markers and genomics in onion breeding. Molecular approaches represented by a combination of different omic technologies including genomics, transcriptomics, proteomics and metabolomics are described as well as future prospects. The resultant effects of these will facilitate the introduction of elite genes that could provide resistance to pests and diseases, and lead to generation of high quality traits.

Introduction

Over 80 million tonnes of onion are produced worldwide (FAOSTAT, 2013). A third of onion production takes places in developing countries and almost all the world population uses onion in their daily meal as a vegetable or as part of a prepared sauce. The average yield potential of onion varies markedly with local growing conditions depending on the locality. Onion is a valuable vegetable crop preceded on the list of worldwide cultivated vegetable species only by tomato in terms of annual production (FAOSTAT, 2011). The bulbs are a globally important dietary source of vitamin C, food fibers, folic acids, and flavonoids serving the plant as both storage organ and a vegetative propagation system.

Onion is a largest member of the Amaryllidaceae, a large herbaceous perennial and bulbous family with more than 57 genera and 500 species (Frodin, 2004). It is the oldest cultivated and most used flavoring vegetables for centuries. The onion crops are widely distributed in developing countries many wild species, which can be crossed directly with cultivated onions, contain a wide continuum to resistance to pest and diseases maintaining and improving crop yields (Chuda and Adamus, 2012). As a vegetatively propagated, highly heterozygous, diploid crop, onion cultivars are often limited in reproductive fertility, making onion breeding a challenging effort. Conventional improvement system compromise relatively long breeding cycles and are dictated by genetic complexity and the sensitivity of onion to inbreeding depression. Plant omics and biotechnology has undergone a revolution over the past decades (Ortiz, 1998; Takayoshi et al. 2005; Langridge and Fleury, 2011). The influences of omics and biotechnology have provided unparalled opportunities for plant production and quality improvement (Adam, 2009).

Plant omics and technology involve the use of cells and tissues by removing, moving or transferring genes of interest for crop improvements. In this paper, we began with the application of cell and tissue culture, reviewed on recent developments and new approaches of omics and biotechnology on onion production and quality improvement. The diverse approaches with central examples instead of providing a thorough survey of the scientific literatures are provided in this review.

Historical Viewpoint of Onion Omics and Biotechnology

Omics and biotechnology in the framework of onion breeding

From the view point of global need of vegetable crops, onion breeding has been highly fruitful over the past century. Traditional breeding methods for onion involve mass selection, hybrid and synthetic varieties development. Single plant derived replica are vegetatively breeded and are assessed for pertinent agronomics and quality traits during repeated seasons of selection. This general approach to onion breeding has resulted in the development of many good clones, which have become highly successful onion cultivars. Despite rigorous breeding efforts, the yield potential of onion cultivars has remained relatively constant over centuries. The main objectives of onion breeding program have involved the increased disease and crop resistance. In addition, improving or maintaining quality traits such as a bulb color, shape, quality and yields and also enhance storability. The significance of historical interspecific hybridization to onion breeding is exemplified by the fact that more than half of all onion cultivars have introgressive

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hybridization from *Allum roylei* stearn. Some cultivars contain introgressive hybridization from more than one uncultivated species (de Vries et al. 1992).

Conventional breeding of diploids plants often requires screening and back-crossing of a large number of plants to achieve the desired genotype. Initial selection of many desirable traits can be ineffective and/or time consuming. Hence, onion breeders often have to screen up to a million seedlings to identify a single clonal line that survives through to the release of a successful cultivar.

In recent past, plants omics and biotechnology have contributed priceless solutions to some of the difficulties associated with onion breeding (Zheng et al. 1999; Zheng et al. 2005). These have related primarily to application of cell and tissue culture. Modern tools of plant transformation and DNA markers offer new opportunities for gaining improved efficiencies in selection of clones of interest and improving genetics as time goes on.

Ploidy manipulation

Ploidy manipulation is considered as a valuable tool in genetic improvement of many crop plants including *Allium species* (Jakse et al. 2003). Haploid production was the first type of ploidy manipulation carried out by Dr. pelaquin and co-workers (Ortiz et al. 2009). The generation of haploid onions was demonstrated through the use of unpollinated ovaries in onions (Muren, 1989). Following this major breakthrough, haploids regeneration has been improved using different media compositions (Campion et al. 1992; Jakse et al. 1996; Martinez et al. 2000; Ponce et al. 2006). A number of factors including genotypic role, heritability of high genogenic responsiveness, the chromosomes doubling efficacy and the male fertility of double haploid line have resulted in the progress realised. Haploid onions are desirable for the study in less complex and laborious backgrounds (Jakse et al. 2010). Such protocol is achieved relatively easy in onion and provides a means to simplify the regeneration of traits from new sources of genetic variety**.**

Embryo rescue

Embryo rescue is referred to as embryo culture (Bridgen, 1994). Plant breeders need access to the largest possible genetic variability. In few instances, variability available within a species is not sufficient to solve a specific problem like disease resistance. The first crosses between *Allium roylet* and *Allium cepa* were made in 1985 to achieve introgression of downy mildew resistance into onion (Van der Meer and de Vries, 1990; Chuda and Adamus, 2012). An extensive study on interspecific hybridization and downy mildew resistant led Scholten and co-worker (2007) obtain FIBCS-generation plants anticipated to be resistant to *Peronospora destructor*. An extreme example of the use of embryo rescue technique was the work of Chuda and Adamus (2012) which reported the interspecific crosses from *A. roylet* into *A. cepa*.

Protoplast regeneration and fusions

Experimental studies on *Allium* protoplast culture have been reported (Vander valt et al. 1990; Fellner, 1993; Buitveld and Creeemersmolenaar, 1994; Fellner and Havranek, 1994; Schum et al. 1994; Ayuba et al. 1995). Buitveld and Creemers-Molenaar (1994) reported successful plant regeneration at low frequency using embryonic suspension derived protoplasts of *A. porrum*. Onion may adapt to this system and can be used to regenerate and transform a competent cell types. Researchers used this technique to culture protoplast from *A. cepa* for plant improvement (Karim and Adachi, 1997). Protoplast electrofusion technique was applied for the production of somatic hybrid plants between Japanese bunching onion (*Allium fistulosum* L.) and bulb onion (*Allium cepa* L.) (Shimonaka et al. 2002). The success of these previous works, onions with improved traits, pests and diseases resistance can be developed.

Somaclonal variation

Larkin and cowcroft (1981) described somaclonal variation as a spontaneous variation of plants observed among those regenerated from cell and tissue culture. Explanation that accounts for the phenotypic changes among somaclonal onions lines might involve the epigenetic or genetic changes associated with cell culture and short regeneration phase of plant transformation. Since not all variety have a heritable bases, line exhibiting phenotypic changes need to be grown on several field season to ensure stability of performance during gene transfer study. Stable phenotypic change of either genetic or epigenetic origin may rise through ploidy level, donor genotype, medium composition and environmental conditions (Veilleux and Johnson, 1998)**.** Recently, Osman and colleagues (2012) reported the application of a molecular marker known as random amplified polymorphic DNA (RAPD) in analyzing and revealing small difference in phenotypic variation among in-vitro regenerated onion plants. Though, it has been recognized overtime, that the recovery of a somaclonal line having negative qualities is very rare. Recently, the phenomenon of somaclonal variation is widely seen as an inherent negative feature of regeneration from cell culture and considered as something to be avoided (Barrell et al, 2013). Approaches to minimize the impact of somaclonal variation are routinely implemented during omics and biotecthnology application of cell culture for onion improvement.

Resistance to pest and diseases

Onion plants are prone to various pests and diseases caused by fungi, bacteria, virus, insects and parasitic nematodes that have negative impacts on yield and bulb quality (Martin et al. 200). While rot disease of onion is one of the most serious fungal disease that affect the roots and the bottom of the bulb and diseased root, loses strength and may completely fail (Goszczynska et al. 200). For these reasons, many approaches are being used to control white rod disease of onion, extending from fungicides to engineering of resistant onion lines (Sammour et al. 2011). Therefore by using genetics and molecular approaches, a number of relevant genes are being identified and new information emerges. Among the genes are oxalate oxides (OXO) genes which are produced by *Sclerotium cepivonum* during hyphal penetration and a synthetic maganin (mgd) gene that produce antimicrobial peptides which may be capable of destroying *Sclerotium cepivonum* hyphae during infection (Eady et al. 2000).

Somatic hybridization

During the past centuries, substantial progress has been made in development of hybrid plant via fusion of somatic protoplast of two different plant species. Somatic hybridization can be stimulated by mechanical and enzymatic treatment depending on the regeneration of hybrid plants. Somatic hybridization can also be used to transfer genes from one specie to another, especially in those cases where symmetric hybrids are produced. Such somatic hybrid plants offer new sources of germplasm for the introgression of traits into crop plants, though this is often very challenging as a result of the poor fertility of the initial somatic hybrids.

Somatic hybridization has provided new opportunities for the production of novel inter specific and intergenic hybrid (Melchers et al. 1978). It also aids gene transfer for pest and diseases resistance and many other quality traits, and

introduction of unique hybrid of nucleus and cytoplasm (Yamashita et al. 2012)

Gene Transfer via Genetic transformation

Genetic transformation is a reliable and rapid technique that can enhance improvement of onion by enabling the introduction of desirable and commercially important genes or traits into known genotypes, without altering their existing, highly selected genetic background. The area of gene transfer in to plat has made substantial and significance progress since the report of regeneration of transgenic plants using *Agrobacterium tumefaciens*- mediated transformation protocols (Horsch et al. 1984; Paszkowiki et al. 1984; Gasser and Fraley, 1989). However these methods initially did not work on monocotyledons plants because the plants were not natural host of *Agrobacterium* (Komai, 2008). Methods like particle bombardment (Sanford et al. 1987), protoplast, electroporation (D'Halluin et al. 1992), silicon carbide whiskers, macroinjection, polyethelene glycol (PEG) mediated (Paszkowski et al. 1984). Ultrasound and pollen mediated have been developed to deliver foreign genes directly into monocotyledonous plants. Spencer and co-workers (1992) demonstrated that direct DNA delivery techniques frequently produce transformant that contain multiple copies of the transgenes and truncated transgenes. Finally, a monocotyledonous cell types co-cultivated technique was developed and *Agrobacterium tumefaciens*- mediated transformation protocol were established for rice by Hiei and colleagues (1994). Therefore many effective transformation protocols in other monocotyledons plants using *Agrobacterium* have been reported. The first routine and stable transformation system of onion was first developed fourteen years ago by Eady and co-workers (2000) using immature embryo of *A. cepa* via *Agrobacterium tumefaciens*. Many developments were made after this breakthrough (Zheng et al. 2001a; Khar et al. 2005; Aswath et al. 2006; Eady et al. 2008).

A stable transformation protocol was developed using calli derived from mature embryo, apical and non-apical root segments of onions and other related species via two different *Agrobacterium tumefaciens* (Zheng et al. 2001a, 2001b, 2004, 2005). A researcher in India reported that in onion, callus proved to be the best expert source for genetic transformation; followed by the shoot tips and root tips (Khar et al. 2005). As a result of the effort made in developing a consistent transformation system for onion and related species, *Bacillus thuringiensis* resistance gene have been produced which confer resistance to beet armyworm in transgenic shallot and garlic plants (Zheng et al. 2004, 2005). Moreover, transgenic plants containing herbicide resistance and antisense versions of allinase genes were reported (Eady, 2002). Transgenic plants tolerant to herbicide containing active ingredients glyphosate and phospinothricin were recovered from immature embryo of open pollinated and hybrid parent was reported (Eady et al. 2003). Aswath and co-workers (2006) derived a new selection system for onion transformation that does not require the use of antibiotics or herbicides using *E. coli* gene that encodes phosphomannose isomerase (pmi). Eady et al. (2008) were able to develop tear-free Onion via single genetic transformation by suppressing the lachrymatory factor synthase gene, using RNA interference silencing.

Omics Technology

Genomics, transcriptomics, proteomic and metabolomics are accelerating at an impressive rate. These tools provide new and unprecedented opportunities for generating new insights and knowledge for crop plants production, food science and biotechnology.

Genomics- based approach

Agronomically important traits or genes may be identified and targeted to produce more nutritious and safe food. The main purpose of the application of genomics is to gain a better understanding of the whole genome of plants. Genetic map development in onion and other *Allium* has been limited by difficulty in developing, preserving and exchanging genetic stocks, high extends of heterozygosity, and a dearth of sequence data (McCallum, 2007).

The typical goal in sampling sequence variation is to detect variants for diagnostic and/or functional analysis, most generally single-nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (indels) (Baldwin et al. 2012). The genomes of onion and related Allium crops such as garlic (*Allium sativum*) and bunching onion (*Allium fistulosum*), are very large (10–20 Gbp) and even transcriptome sequencing has been limited to modest expressed sequence tag (EST) projects (Kuhl et al. 2004; McCallum et al. 2001). A partial (0.3N) onion bacterial artificial chromosome (BAC) library (Suzuki et al. 2001) provided insights into gene structure and genome composition, most especially the very low gene density of one gene per 168 kb (Jakse et al. 2008). The initial genetic linkage map 'BYG15-23 x AC43' developed by Havey and colleagues using Restriction Fragment Length Polymorphisms (RFLP) markers remains the key reference map (King et al. 1998; Bradeen and Harvey, 1995; Martin et al. 2005). The first published genetic map of an Allium species was that developed by King and co-workers (1998) in the intraspecific onion cross 'BYG15-23 x AC43' (McCallum et al. 2012). Notably, this map revealed a very high degree of dominant r*estriction fragment length polymorphism* (RFLP), proposing that the large genome size of onion is associated with high degree of gene duplication (Baldwin et al. 2012). Genetic stocks used to date for development of onion mapping populations are generally inbred lines that have typically been only subjected to one generation of self-pollination. The high levels of remaining heterozygosity have earlier greatly complicated marker development and sequence analysis in onion. Though few researchers have produced doubled haploid onion (DH) lines, these have in general suffered from poor seed set (Bohanec, 2002). The development of highly fecund DH lines from long-day US onion varieties by Alan et al. (2004, 2003) now provides chance to exploit the use of homozygous and distributable reference lines for onion genetics and genomics.

Transcriptomics- based approach

Tremendous technical advances of the post- genomic era, data is no longer a limiting factor in advancing biological research. Transcriptomics, also called global analysis of gene expression or genome-wide expression profiling (Zhang et al. 2010). Baldwin et al. (2012) developed PCR-based genetic markers that were easily transferable among the *Allium* research and scientific community, based on transcriptome sequence polymorphisms segregating in a wide bulb onion cross. This information provides a framework for genetic analysis and genome sequencing in onion. Duangjit and co-workers (2013) reported a transcriptome sequencing to produce SNP-based genetic maps of onion using the Roche-454 platform to sequence from normalized cDNA libraries from two inbred lines of onion. The ability to easily develop custom panels of SNP markers for interrogating genes or genome regions of interest will complement modern genetic strategies that identify

candidate variants through deep sequencing of population samples.

Proteomics- based approach

Proteins are vital parts of living things, as they are the major components for building the cellular structure and they also function as catalytic enzymes and signal transduction proteins involve in metabolic and regulatory pathways of cells respectively (Graham et al. 2007). The analysis of protein is the most direct approach to define a function of its associated genes. Though, it must be mentioned that the genome and proteome of an organism do not always correspond to each other on a one-to- one basis (Komatsu, 2007; Schroeder et al. 2001). The ability to identify and measure protein molecules on a large scale depends on recent advances in high-throughput proteomics techniques, which aim at the simultaneous analysis of all proteins expressed by plant cell or tissue in a specific condition. The term proteomics was coined to make an analogy with genomics and transcriptomics as a tool for the large-scale study of proteins, particularly their functions and structures (Wilkins et al. 1996; James, 1997). In some monocotyledons like rice, stress- induced proteins were investigated using different tissues and organelles.

Metabolomics- based approach

Metabolites are the end product of cellular process and they reflect the response of the biological system (Royuela et al. 2000). The current trend in metabolic studies is to define the cellular status at a particular time points by quantification of the total metabolites in the cellular system (Hollywood et al. 2006). These techniques in conjunction with genomics, transcriptomics and proteomics could portray exact pictures of the whole cellular processes and give more insight in onion breeding. A range of analytical technology is available for plant metabolome study (Okazaki and Saito, 2012), as well as high through put approaches such gas chromatography- mass spectrometry (GC-MS) (Kaplan et al. 2004), ultrahigh- resolution Fourier transform- ion cyclotron MS (Hirai et al., 2004), Fourier transform infrared (FT- IR) (Johnson et al. 2003), and nuclear magnetic resonance (NMR) (Kim et al. 2010). Time effect, drought, heat, stress and environmental condition have been studied with these techniques and hundreds of metabolites were examined simultaneously (Dixon et al. 2006). Recently, several mainstream and specialized techniques of plant metabolomics were reported (Allwood et al. 2011). This huge amount of information may be utilized to improve crop breeding and production. Hundreds if not thousands of biosynthetically-related metabolites and enzymes could be analyzed by metabolic profiling instead of monitoring a single putative gene which in turn could be used to develop stable genotypes for crop cultivars (Dixon et al. 2006). In single plant species, a few thousand secondary metabolites may be observed easily during metabolic profiling (Saxena and Cramer, 2013).

Prospect

Onion plant is an important source of vitamin C, food fiber, folic acids, and flavonoids Bhattacharjee et al. 2013). In many countries, including Africa, onion plays a key role in the diet and income of some millions of people. High yields, bulb color and shape, storability, pests and diseases resistance have been the breeding objectives for onion.

Through applications of biotechnologies such as tissue and cell culture, genetic engineering, marker-assisted technologies, genome-assisted technologies or a combination of all the technologies for the improvement in potato described in this review, with this kind of techniques, possibilities for improving these traits are limitless. Indeed biotechnology is yet to be applied successfully to the improvement of onions anywhere in the world including the developed countries. To increase onion production and improvement through biotechnology, the following directions for research in omics and biotechnology of this crop plant are suggested:

Ploidy manipulation is an alternative technique to induced haploid regeneration in onions (Ponce et al. 2006). More haploid regenerant should be produced by ploidy manipulation to improve onion for breeding purpose.

Embryo rescue techniques have enabled successful intergeneric and interspecific hybridization for centuries (Ramming, 1990). Hybrid embryo developments have been developed through embryo rescue. These hybrids could have horticulturally and agronomically important traits.

Protoplast regeneration and fusions have been used to improve a plant's agronomic and horticultural characteristics such as pests and diseases resistance (Shimonaka et al. 2002). This technique should be used to produce more somatic hybrids that are pests and diseases resistant.

Somatic variation can leads to the creation of additional genetic variation in onion. Herbicides and tolerance to environmental or chemical stress have been developed via this technique (Brown et al. 2006). This technique could be used to introduce horticulturally and agronomically important genes from onions.

Somatic hybridization is an alternative technique to overcome both intraspecific and interspecific cross incompatibility to a large extent (Barrell et al. 2013). More hybrids should be produced by somatic hybridization to enrich onion germplasm for breeding use.

A. tumefaciens-mediated transformation techniques have been established using embryogenic suspension cultures of onions (Zheng et al. 2005). Introduction of horticulturally and agronomically important genes to commercial onion cultivars should be conducted in a large scale.

High-density genetic linkage maps should be constructed. Molecular markers tightly linked to agronomically important traits or genes can be developed and utilized in the selection of new cultivars. Quantitative trait loci (QTLs) for introgression breeding and whole-genome approaches should be mapped and cloned using molecular tags such as SNPs. A further goal for the near future should be to obtain the complete sequence of the onion genome.

Access to these biotechnologies is of vital importance for developing countries. Private and public funding and cooperative research to ensure that the benefits of omics and biotechnology to improve onion yield and productions are extended.

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