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Review Paper

Role of peptide hormones in plants

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Abstract

The classical plant growth regulators have been studied as key regulators in the growth and development of plants since nineteenth century, but the researches of last few years indicate that peptides are also take part in plant signaling for growth and developmental processes like defense responses, cell elongation, cell differentiation, cell proliferation, meristem organization, nodule development, self incompatibility and organ abscission etc. In plants, peptides are synthesized by using mRNA as a template and most often go to post translational modifications to yield mature peptide. Here in this review paper we are trying to provide an overview on peptide hormones along with their functions regarding plant growth and development.

Keywords: Classical plant growth regulators, plant signaling, post translational modifications, peptide hormones, plant growth and development.

Introduction

Signal transduction plays a vital role in the development of multicellular organisms (whether they are animal or plant) and also so for in the functioning of various organ systems. In plants cell signaling is mostly mediated by classical growth regulators i.e. auxins, cytokinins, gibberellins, ethylene and abscissic acid[1]. Later on, the list of these growth regulators is upgraded by adding brassinosteroids, jasmonates, salicylates, strigolactones and karrikins[2-3]. Research findings of over the last few decades indicate that beside these plant hormones other molecules, including peptide hormones (also known as signaling peptides), transcription factors as well as sRNAs are also have significant roles in signaling[2,4-5].

Peptide hormones are now widely accepted as signaling messengers in plants for their involvement in regulation of several growth and developmental processes like defense responses, self incompatibility, callus growth, nodule development, root growth, organ abscission, meristem organization and regulation of leaf shape etc. The identification of majority of these plant peptide hormones has been taken place through biochemical and genetic studies[1].

Large molecular weight precursors frequently processed by proteolytic cleavage to produce active form of peptide hormones. Plant peptides are water soluble and active in the nanomolar to picomolar range[6-7]. The first peptide hormone was recognized in 1991 but the information on peptide hormones in plant is meager as compared to the information available for the peptide hormones in animals. So in this review paper some peptide hormones are discussed with their importance in biological processes of plant development and growth.

Structural characteristics and biosynthetic pathway

On the basis of structural characteristics the plant peptide hormones are classified into two groups i.e. cysteine poor and cysteine rich peptides. The cysteine poor peptides are also known as small post translationally modified peptides. Like other proteins, peptide hormones are also synthesized by translation in which mRNA is used as a template. The precursors for peptide hormones are processed either inside to the endoplasmic reticulum or sometimes may processed outside of the cell. In addition to this sometimes these are also modified by the processes of glycosylation and hydroxylation[8]. The secreted peptide encoding genes are transcribed first and after that they are translated as pre-propeptides and then pre-propeptides produce propeptides. During this process signal peptidase removes the N terminal signal peptides. Further modifications of these propeptides by numerous enzymes are resulting into the production of mature and functional peptides[9]. However based on their biosynthetic pathway these signaling peptides are categorized into the three major groups in which first group is of cystein poor peptides, second group is of cysteine rich peptides while the third group is of intermediate of both so known as intermediate type peptides[5,10] (Figure-1).

The first group of these peptide hormones contains small peptides resulting from the post translational modifications. So these peptide hormones are also known as small post translationally modified peptides. The members belonging to this group are cysteine poor peptides having amino acids less than 20. The propeptides for these mature peptides are known to contain amino acids about 70 to 120. These are always the

product of the proteolytic processes and contain a C-terminal conserved motif which mostly leads to proline residues as well as post translational modifications[9,11]. Most of the peptide hormones of plants are belonging to this group such as PSK, CLV3/ESR, PSY1, CEP and RGF/GLV/CLEL[12-22]. The second group of secreted peptides contains the peptides rich in cysteine. The main characteristic feature of these peptides is that they are consists of an even (usually six or eight) number of cysteine residues. The 3-D structures of mature proteins are determined by the intramolecular disulfide bonds. The functional signaling peptides of this group are usually having large size usually more than 20 amino acids[9,11]. The cysteine rich peptides include the SCR/SP11 and LUREs[23-25]. The third group of the signaling peptides is commonly referred as the intermediate type peptides, is intermediate between above mentioned both groups. The signaling peptides belonging to this group may be like both previously discussed groups i.e. contain intra-molecular disulfide bonds like cysteine rich peptides and may also be proteolytically processed like cysteine poor peptides[9]. Stomagens and RALFs are the representative of this intermediate-type peptide group[26-28].

Post translational modifications

Post translation modification process takes place for maturation of small cysteine poor peptides. Mainly small peptides of plants are post translationally modified by the involvement of tyrosine sulfation, proline hydroxylation and hydroxyproline arabinosylation.

Tyrosine sulfation is a process in which the sulfonate moiety of 3'-phosphoadenosine-5'-phosphosulfate transfers the hydroxyl group of tyrosine residue[29]. The process is catalyzed by tyrosyl protein sulfotransferase enzyme, which is located in golgibody. Tyrosine sulfation is proteolytic processing of bioactive peptides which modulate the function of proteins throughout the multicellular organisms like as extracellular interaction of proteins[10,30]. PSK, PSY1 and RGF1 are the identified tyrosine sulfated peptides in plants[16,20,31].

Proline hydroxylation is a process by which a non-proteinogenic amino acid hydroxyproline is formed in which hydroxyl group attached to the gamma carbon atom of proline. The process is catalyzed by the prolyl-4-hydoxylase (P4H) enzyme, which is localized in the lumen of endoplasmic reticulum and golgiapparatus. Prolyl-4-hydoxylase enzyme is a member of 2-oxoglutarate dependent dioxygenases family, which comprises N-terminal transmembrane domain[32]. Proline hydroxylated peptides have been identified in plants are TDIF, CLV3, PSY1 and RGF1[13,16,17,20,33].

Hydroxyproline arabinosylation is catalyzed by hydroxyproline O-arabinosyltransferase (HPAT) enzyme. In this process L-arabinose is transferred to the hydroxyl group of peptide bound hydroxyproline residues which is catalyzed by the enzyme HPAT[34]. HPAT enzyme is a transmembrane protein located in golgibody and structurally resembles to the GT8 family member glycosyltransferase. Three hydroxyproline O-arabinosyltransferase genes are present in *Arabidopsis thaliana* genome, out of which (HPAT3) plays an important role in arabinosylation of CLE peptides[9,34].

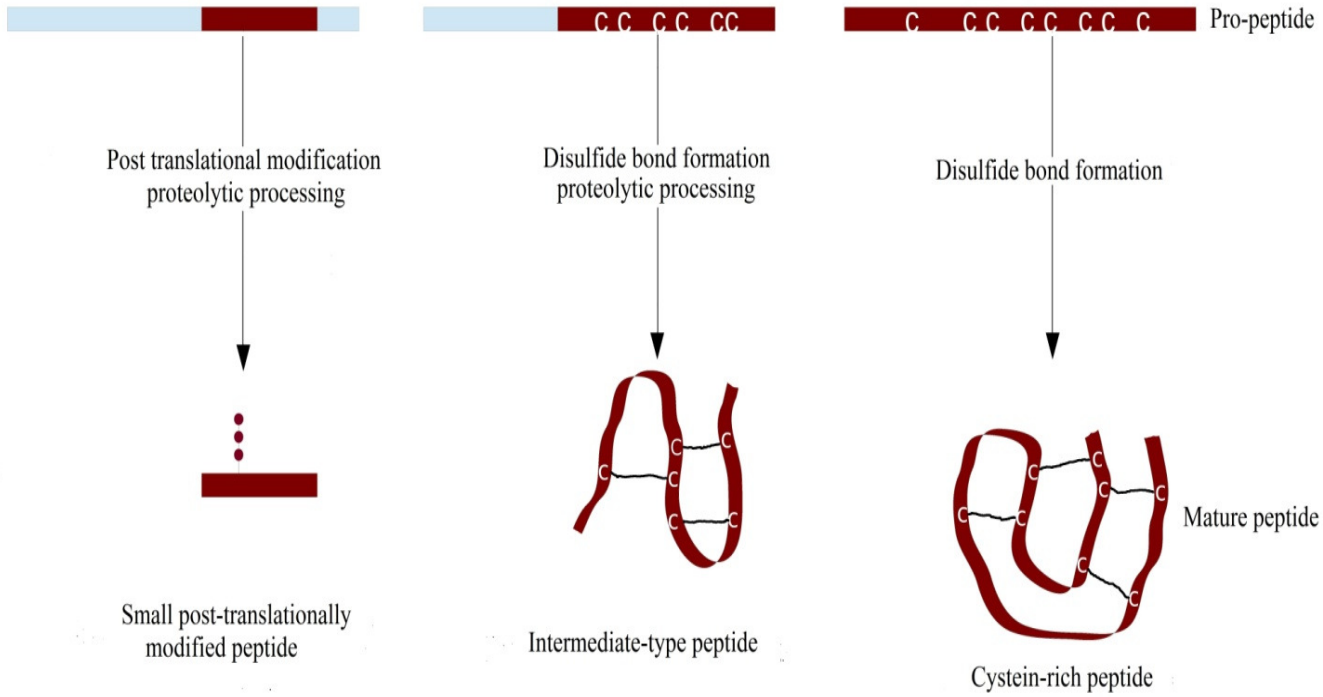

Figure-1: Different types of signaling peptides and their maturation[9].

Some peptide hormones and their roles during growth and development in plants

Systemin: During the insect attack many plants secrete some defense proteins responding to wounds in the leaves and stems of some solanaceous plants like tomato *Solanum lycopersicum* and *Solanum tuberosum*[35]. In leaves of these plants, the defense proteins (i.e. protease inhibitor I and protease inhibitor II) was recognized which inhibit the development and growth of feeding insect or pathogen through interfering with their protein digestion[36]. It is found that these defense proteins also accumulate in the unwounded leaves far away from damaged sites along with wounded leaves. This suggests that this systemic defense response is induced by a long distance signal transmission. The factor involve in this long distance signaling was isolated from the tomato leaves and named as systemin (TomSys due to isolated from the tomato)[6]. Systemin consists of 18 amino acids and was the first isolated peptide hormones in plants. Many research findings indicate that the exudation of TomSys into vascular bundles from the damaged sites stimulates biosynthesis of jasmonic acid in enclosing vascular tissues which thought to be worked as long distance signaling messenger including systemic wound response by producing defense proteins at the target site or in unwounded leaves[37].

The tomato systemin (TomSys) precursor is a 200 amino acid polypeptide resulting from the transcription[38]. This TomSys precursor does not have a putative signal sequence so it is suggested that this precursor is synthesized on ribosomes particularly in the cytosol[39]. In unwounded tomato leaves mRNA encoding systemin precursor is present in very small amounts but accumulates upon wounding mainly in the sieve tube elements surrounding cells of phloem tissues in the vascular systems of the mid veins. After wounding, systemin precursors are stored solely in the cells of phloem parenchyma of tomato leaves.

The membrane localized receptors perceive the environmental, pathogenic and hormonal signals to activate functions leading defense responses, plant growth and development by transducing signals inside the plant cells. In fully sequenced genome of *A. thaliana* approximate 600 or more RLK genes have been recognized in which approximate 200 RLK genes are belonging to a receptors family known as LRR-RLKs. Despite the large number of LRR-RLKs in plants, approximate 10 of which have well known their biological functions like meristem signaling, hormonal perception and pathogenic responses[40]. A LRR-RLK known as SR160 has been reported during some studies in tomato as systemin receptor[41].

Systemin plays a very crucial role during the defense signaling. It enhances the biosynthesis of more than 20 proteins belonging to defense, mainly proteases, proteins related to signaling pathway and antinutritional proteins in tomato[36]. Prosystemin over-expression induced a significant reduction in larvae damage which indicates that the great deal of constitutive protection is much more towards the inducible defense mechanism[42]. Though in tomato plants, the continuous prosystemin activation is not only expensive but also have an important impact on the growth, developmental, physiological and reproductive processes[43]. The protease inhibitors production was mostly reduced due to systemin silencing in tomato which also results three time faster growth in larvae feeding on the plants[44]. Over expression of systemin has been observed with a stimulating effect in the tolerance of plants to various abiotic stresses like ultra violet radiation and salt stress[45]. During the salt stress systemin transgenics plants are resulting with low concentration of proline and abscissic acid in leaves but have higher biomass and stomatal conductances. These studies indicating that systemin allows the plant to either they maintain themselves to the salt stress conditions more competently or they may perceived a comparatively less stressful environment[45].

When plants parallel grown under ultra violet B light and normal condition (without ultra violet B radiation) it is found that plants exposing ultra violet B light are shown more resistance towards the insect herbivory comparatively to the plants growing under normal condition. Exposing a tomato plant with ultra violet B radiation is resulting in the accumulation of weakly wounded protease inhibitors in whole plant. However, in case of solely radiation or weak wounding by themselves is not capable of inducing the accumulation of systemic protease inhibitors in tomato. In cell culture in tomato, MAPKs are activated by both systemin as well as ultra violet B performing jointly. A short pulse of ultra violet B also causes alkalization in the cell culture medium[37,45]. Beside these, systemin also reported to induce root growth in some species like *Solanum pimpinellifolium* clearly indicating that systemin have also contains a significant role in some plant growth and developmental processes[46].

Phytosulfokine (PSK): As in normal cases fully differentiated mesophyll cells proliferation is suppressed by low cell density, but the growth of these fully differentiated mesophyll cells was considerably enhances by addition of a conditioned medium which was found in *Asparagus* cell culture[32]. This bioassay was used to purify an active factor from that conditioned medium. The purified factor was identified and observe that it is sulfated peptide consists of 5 amino acids. This peptide was named as phytosulfokine due to the presence of these sulfated easters[32]. A ~80 amino acids precursor produces phytosulfokines during the enzymatic processes which has secretion signals at N terminal[46]. Precursor genes of phytosulfokines are found unnecessarily dispersed throughout the genome in the condition medium resulting from the cell line of many angiosperms and gymnosperms plant species including *Asparagus*, rice and maize, *Zinnia*, carrot and *Arabidopsis*, which shows that phytosulfokine is a widely distributed peptide hormone among the higher plants[31,48-52].

Phytosulfokine containing two post translationally sulfated tyrosine residue and derived from the cleavage of the precursor proteins. Tyrosil protein sulfotransferase catalyzes the tyrosine-

sulfation of pre-prophytosulfokine in the golgi apparatus[52-53]. On the basis of some research findings it is suggested that the LRR-RLKs are the part of the active functional phytosulfokine receptors that directly interact with phytosulfokine[54]. Phytosulfokine binding leucine rich repeat receptor like kinase is known as PSKR1. Though the PSKR1 expressions have been noticed in the whole tissues of hypocotyls, apical meristem, leaves and carrot root seedlings, but comparatively higher expressions of PSKR1 have been noticed in the cultured carrot cells[31].

Rapid Alkalinization Factor (RALF): When experiments were carried out on tobacco in the search of systemin, an unidentified peptide factor composed of 49 amino acids was noticed that causes the alkalinization of suspension culture mediums even faster than that of systemin but does not activate defense response[55-56]. So due to the ability of rapidly alkalinizing the suspension culture medium this new discovered factor was named as RALF. Beside tobacco, highly conserved similar sequence has been also identified in alfalfa and tomato leaves[56]. On the basis of tomato sequence a peptide sequence was synthesized and it was noticed that native as well as synthetic both are active at very low (in nanomolar) concentrations. However, the synthetic peptide was inactivated by the alkylation of reduced peptides that breaks to the disulfide bridges formation[57].

RALFs are considered to be involved in plant defense responses as RALFs and systemins both induce the alkalinization of the medium of MAP kinase activation[55]. On the basis of the intentional RALF genes the specific aspects or overall growth and development of plants can be inhibited by the over-expression of the RALF genes. In *Arabidopsis thaliana* during the transgenic studies it was found that the semi dwarf plants were resulting from the over-expression of either the *AtRALF1* gene or the *AtRALF23* gene[58-59]. In *Madicago trunculata*, a RALF gene (*MtRALF1*) was found which was expressed by the roots and upregulated by the nodulation factors[60]. During the transgenic studies in *Madicago trunculata,* the over-expression of the *MtRALF1* gene was found to be involved in nodule reduction and an enhancement to the terminated infection threads. So, all of these studies suggest that RALF peptides are the key factor in the negatively cell growth regulation, particularly through the cell expansion inhibition[57].

CLAVATA3 (CLV3): Plants are the representative of the persistent growth in the above-ground aerial parts due to the continuous activity of the shoot containing meristematic cells known as shoot apical meristem (SAM). Based on their functions SAM categorized into three different zones: peripheral zone denotes as PZ which forms the lateral organs of plants; rib zone denotes as RZ which forms stem core of plants and the last central zone denotes as CZ which is known for slow cell division. CZ is the main source for the cells of the peripheral zone and rib zone. In plant SAM, it is mandatory to maintain the balance between the central zone (stem cells) and peripheral zone (differentiating cells). The genes responsible for the maintaining these balances in the cells of each zone are known as CLV genes[8]. The CLV genes are named after a latin word *clavata* which means club shape because the floral meristems in CLV mutants produce many extra club shaped carpels[1]. In *Arabidopsis* the mutation in CLV1-3 genes are leads to the loss of functions which results to modifications in the inflorescence architecture through the enlargement in the size in the SAMs[61-62].

In 1995 a gene was reported to be responsible particularly for the regulation of floral meristem and shoot meristem that was identified and named as the CLV3 gene. Fletcher and his coworkers identified CLV3 gene as the signaling peptides in plants. It is expressed in the stem cell layers especially in the L1-L3 layers of central zone and in deeper cell layers of L3 only CLV1 is expressed. This leads a suggestion that the secretion of CLV3 peptides could be taken place from stem cells and are produced and attached to CLV2/CRN or CLV1 receptor complex in the L3 cells[12]. It is suggested that CLV3 genes are extra-cellular signaling polypeptides which are the main factors for determination of cell fate in SAM, but their chemical structure is unknown[63-64].

The CLV proteins have an important role in WUS expression restriction of stem cell domains. Therefore, overgrowth caused due to the WUS over-expression resulting in the plants that is much resembles with *clv* mutants[65]. Few CLV3 peptides which are synthesized chemically like CLV3A, CLV3B, CLV3L, MCLV3 and MCLV are known for reduction in RAM and SAM size, this is based on the fact that CLV3 genes over-expression resulting in the reduction of RAM and SAM size[8]. The reduced RAM and SAM size indicates that CLV like signaling pathways activation might also be regulate the RAM growth and cell fate within the roots[1,66].

Early Nodulin40 (ENOD40): In most leguminous plants a nitrogen fixing bacteria commonly known as *Rhizobia* is take part in the formation of root nodules. *Rhizobia* involve in the induction of nodule development in leguminous plant roots by producing the lipochito oligosaccharides known as nod factors. Noduline genes are classified into two classes first as ENOD (stands for early nodulin) genes and second as LNOD (stands for late nodulin) genes. Among polypeptides in plants, ENOD40 was the first polypeptide deduced by gene analysis and overall second polypeptide to be identified in the plants[56]. ENOD40 is suggested to encode the peptides consisting of 9 to 24 amino acids[67]. *Rhizobia* induce the nod factors to stimulate ENOD40 induction well before the beginning of cell division in the root cortical cells which is strongly suggested that the ENOD40 is actively participated in nodule development in legume plants. However, ENOD40 homologs have also been identified in the nonleguminous plants including monocots respect from leguminous plants suggesting that ENOD40 also have some functional role in growth and developmental processes besides the nodule organogenesis[68-72].

In *Lotus japonica,* the reduced level of ENOD40 stimulates significant reduction in nodule development and its over-expression accelerates nodulation in *Medicago*[73]. Research findings of such studies are suggesting about the importance of ENOD40 as main factor in nodule development. Although, over-expression of ENOD40 has no significant visual changes in plant growth, this indicating that ENOD40 does not involve directly in stimulating the cell division, however it somewhat sensitizes the cells to induce signals responsible for division[1]. Expressions of ENOD have also been observed in non-symbiotic organs in leguminous plants and the homologs have also been observed in nonleguminous[68,74,75]. At an early developmental stage of the lateral vascular tissues in rice ENOD40 is limited to protoxylem surrounding parenchyma cells. This suggests evidently the importance and involvement of ENOD40 peptides in vascular bundle development[67,76]. All these research findings suggesting that ENOD40 is initially involved into another pathway for plant development rather than pathway for nodule development, and then it was employ into the pathways for symbiotic nodulation.

S-Locus Cysteine Rich Protein or S-Locus Protein11 (SCR or SP11): In many flowering plants pistil recognized and rejects the pollens of closely linked individuals to check inbreeding, in this way genetic diversity is maintained within the plant species, this is known as self-incompatibility. It is revealed through genetic studies that a solitary multi-allelic locus which is known as S-locus (sterility locus) controls the self incompatibility[77]. A highly polymorphic, small and anther-specific gene was discovered which is responsible for controlling the pollen functioning in self-incompatibility. This gene was located between SLG and SRK at the S-locus and named as the SCR or SP11[23,78]. Self-incompatibility determinants have been recognized in some species of *Brassica* by the molecular cloning of S locus genes. The products of S locus genes are shown particularly in the pollen, stigma or anther[1].

It is suggested that the product of SCR or SP11 gene is essential and enough for the determination of pollen self incompatibility. Research findings of Immuno-histochemical studies indicated that during early stage of anther development, the secretion of SP11 into anther-locules by the tapetal cells as cluster and then translocated on pollen surface. However during the pollination it is transported to the papilla cell from the pollen surface and then it go through to the cuticle layers to disperse throughout the pectin-cellulose layer[79,80]. The pollens in the members of *Brassica* species altered through a particular SCR or SP11 haplotypes obtained the self-incompatibility precisely determined by the transgenes. When chemically synthesized SCR or SP11 peptides or recombinants are supplied to the stigmas at very low concentration (50fmol per stigma) it resulting in the hydration inhibition of compatible pollen[78].

POLARIS (PLS): The expression of reporter genes mainly in roots was analyzed by the promoter mediated transgenic lines, a new gene was discovered which was identified and named as the POLARIS gene. Expressions of POLARIS have been noticed from the heart stage in the embryonic roots and, lateral and primary root tip in seedlings[81]. A 36 amino acid long peptide residue encoded by a small ORF of auxin inducible transcripts[82]. This suggested peptide of 36 amino acid residue does not have any secretion signal; this indicated that its roles are in the functions related to cytoplasm. However there is lack of such evidence to prove its intracellular localization. There are many research efforts made to isolate PLS peptide but it still has not biochemically isolated[1].

The *pls* mutants are characterized with enhanced radial expansion and decreased cell expansion resulting in a short root phenotypes and decreased leaf vascularization. The roots of *pls* mutants are over reactive to exogenously supplied cytokinins and these mutants show the enhancement in the expression of ARR5/IBC6 (cytokinin inducible genes) in comparison to wild type[76]. Over-expression of PLS decreases the root growth reduction by exogenously supplied cytokinins and enhances leaf vascularization[1].

Inflorescence Deficient in Abscission (IDA): Plants shed their unnecessary parts like old leaves and/or floral organs from the parent plant body by a natural physiological process known as Abscission. Abscission begins with the development of abscission zone that segregates to the parent plants from the parts to be shedding. During the screening for the delayed floral abscission mutants, an *Arabidopsis* mutant called *ida* was identified which retains its floral organs indefinitely[83]. In *ida* mutant plants the senesced dry floral parts live attached, even though the mature seeds are shedding. IDA gene is responsible to encode a peptide consisting 77 amino acid residues with the N-terminal secretion signals. Promoter study has revealed that during the process of floral abscission, the IDA expression is observed in the abscission zone in floral organs. In *Arabidopsis*, the research findings have also been recognized 5 genes that are paralogous to the IDA and IDl 1-5. The sequence alignments of the deduce peptides belonging to this family show the existence of an extremely conserved domain bounded by the residues of basic amino acids close to C terminal[1].

The evidences of past studies suggest that IDA encodes a small signaling peptide which works as an active ligand for the HAESA. This small signaling peptide is *Arabidopsis thaliana* plasma membrane linked LRR-RLK which is known to involve in to the regulation of abscission in floral organs[84]. The HAESA expression is observed not only at the bottom of the petioles and the pedicels but also in abscission zones of floral organs. However the IDA peptides are well-known for their involvement in the abscission of floral organs[83]. But in recent studies a new role of IDA peptides has been observed in the helping to lateral root primordial passage through not only main roots but also assists in lateral roots[85].

ROTUNDIFOLIA/DEVIL1 (ROT/DVL1): During the screening for the activation tagged population of *Arabidopsis*

thaliana to isolate the mutants of leaf shape, a new gene was identified which is known as ROTUNDIFOLIA (ROT4)[86]. While at around the same time another gene was discovered in the activation pool and known as DEVIL1 (DVL1)[87]. Expression of ROT4 genes altered by T-DNA mediated insertion of 35S enhancers which is known as dominant *rot4-1D* mutant. The plants having *rot4-1D* mutants have the characteristics like small rounded leaves, short inflorescence and floral organs. Generally the following characters are expressed by ROT4 gene i.e. reduced cell proliferation and organ development. These studies also suggested that the proliferation of polar cells is also controlled by ROT4 gene. *dvl1-1D* mutant plants have the same phenotypic characters like as *rot4-1D* i.e. rounder leaves, shortened siliques and petioles, and moderately altered shape of fruit tips[1].

The ROT4 genes consist of a 53 amino acid peptide while DVL1 genes consist of peptides of 51 amino acids and these are encoded by ORFs. The sequence of amino acids in both is extremely homologous to one another. In *Arabidopsis* a gene family is recognized which contains 23 genes encoding small peptides and both ROT4 as well as DVL1 considered as the member of this gene family. The phenotype of *rot1-1D* is rescued by the ROT4 and GFP over-expression which suggest that small open reading frame expression is enough to stimulate its functions. ROT4/DVL1 gene family functional redundancy is very consistent to the observations that single member disruption in a family never causes an apparent change in phenotype. Over-expression of both, ROT4 and DVL1 is apparent in leaves, however over-expression of several other members belonging to ROT4/DVL1 family are apparent in roots and flowers[1].

Conclusion

Classical plant growth hormones are well studied in plants than peptide hormones due to their importance in plant growth and developmental processes. However, now it is widely accepted that peptide hormones are also very crucial for intercellular and intracellular signaling in higher plants. In *Arabidopsis* potential signaling peptides were encoded by more than 1000 genes[17,88]. Bio-computational analysis of genomic data would be promising approach to detect the gene responsible for encoding of signaling peptides. There are many aspects remain unexposed about the signaling peptides in plants. Biosynthesis of signaling peptides is not much explored and the information available is very little, only a few proteases have been reported to be involved in maturation processes of the precursors to produce functional mature peptides so it is very hard to explain how they are produced in plants. The initial steps involve in translation in the production of mature signaling peptides is still hypothetical[59,89,90]. The importance of signaling peptide hormones to control the physiology, growth and development in plants through various processes are studied but in a number of cases, signaling peptides binding receptors and their downstream targets are still not known. It is expected that ongoing researches on forward and reverse genetic studies will surely provide some imminent knowledge regarding this in the near future.

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