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NLP phytotoxin, a selective - biological herbicide of dicot weeds

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***Abstract*—** NLPs (Necrosis and ethylene inducing proteins) as microbial phytotoxins have been found in extensive range of microorganisms with different necrotic ability that cause hypersensitive immune response in dicotyledones weeds which result in extensive necrosis and death. This protein is not recognized by the immune system of monocot plants. NLPs have been used on dicot plants with various method like spraying, injection, hanging drop method and transferring its corresponding gene by Agrobacterium bacteria to the plant. Also a lot of auxiliary factors have been done such as the use of detergents, fungal or bacterial cell mid NLPs in order to increasing its effectiveness. The purpose of this paper is review of the target plants or host range of NLP, the range of microorganisms having this phytotoxine, how it works, ways to effectively use it as well as factors affecting its functionality as a biological herbicide.

***Keywords*—**Biological control, NLP, dicot weed, monocot weed

I. INTRODUCTION

Weeds has always been a serious problem for farmers. In order to restricting and inhibiting the growth of the weeds, different strategies have been employed. The biological control methods attract attention, due to having no environmental pollution and production residues. NLP protein was isolated first in 1995 from Fusarium oxyporum [10]. After discovering it, NLPs have been used for biological control of various plants, especially weeds [12], [7], [17]. This protein effectively causes necrosis and induces expression of ethylene hormone, in a large number of dicot plants [7], [8], [24]. Various studies have been done on the mechanism of this protein in plant cells [26], target plants [31], and it producing microorganisms [16]. This protein is stable at ambient temperature for 24 days and since there are a lot of dicot weed, This protein has been introduced as a good candidate in the fields of monocots cultures such as wheat, barley, Zea and so on [15], [12], [5], [8]. It should also be noted that no human toxicity of this protein have been reported [8].

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II. TARGET PLANTS:

The table below shows the most important plants that have been presented for biosensing with this protein. With a careful look at the table it can be concluded that the biological assays is done in most cases on the weeds in the tropics of South America and especially in the field of cocoa [6], [11]. Of course a research was conducted by Moghimi in iran that tobacco, tomatoes, Sophora alopecuriodes, sinapis arvensis has been studied [23], [3] and also Mirzaie [2] has worked on Sophora alopecuriodes and triticum aestivum and Shakeri has studied the effect of this protein on Sophora alopecuriodes, sinapis arvensis, Amaranthus retroflexus, Chenopodium album, Rumex crispus, Alisma plantago aquatic and bread wheat [1].

I. Target plants- [16], [17], [7], [32], [12]

Salvia officinalis	Ocimim basilicum	Opium Poppy	Coriandrum sativum	Ambrosia artemisiifolia	**Erythroxylum coca**
Centaurea maculosa	Abutilon theophrasti	Trifolium repens	Diospyros virginiana	Albizia julibrissin	Nicotiana tabacum
Theobrom	Cirsium	Taraxacum	Cannabis	Sasamum	**Albizia**

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a cacao	arvense	officinale	sativa	indicum	**julibrissin**

III. RANGE OF MICROORGANISMS HAVING NLP GENE:

Many plant pathogens secrete progressing pathogenic bacteria toxins that its role is performed by cell killing. NLP producers like many bacteria, fungi, and oomysets specious establish a leaf necrosis and mediated immunological responses in many plants [25]. Many of these organisms are plant pathogens [19], so presence of NLP in a variety of herbal microorganisms, indicating the important role of these genes in pathogenecity. NLP as a trigger, causing necrosis of leaves, which are genetically distinct from immune-mediated cell death [27] and stimulates dependent protective levels of immunity in all studied dicot plants, But it is unsuccessful in monocot plants [19]. It is interesting that although NLPs are present in distinct organisms phylogenetically but their sequences remain remarkably conserved during evolution [26]. 7 amino acid of GHRHDWE and some Cysteine roots in all the sequences of these proteins are present in different organisms [30] Although protected areas can be related to the strategy of phatogenecity, However, the homologous gene in non phatogens like *Vibrio pommerensi* bacteria and ascomyset fungi such as Neurospora crassa is found. Also no homologue of NLP genes in plants, animals or Protista has been reported till now [33].

IV. ACTION MECHANISM OF NLPS IN DICOTYLEDONOUS PLANTS:

About action mechanism of these proteins and the mechanism of necrosis, numerous studies have been conducted. It has been recognized that NLPs are elicitors (compounds activating plant defense mechanisms) [20]. According to various studies it is suggested that this protein induces a series of responses similar to hypersensitivity in the plants. Hypersensitive responses in the plants induced locally infection and eventually cause necrosis of the infected area which results in higher expression of ACC synthase and ACC oxidase enzymes, these enzymes are key products of ethylene biosynthesis. Following the increase in ethylene, activation of MAP kinase, phytoalexine synthesis and intracellular calcium increase is observed [6]. As regards the mechanisms of immune responses is induced by plants to limit the infection process, but the severity of these responses cause the loss of plant. In the other words, NLPs Log in vascular plants and induce plant defense responses lead to signaling cascade in plant, resulting in extensive tissue necrosis and death [4]. The answers can cause extensive necrosis usually in 24 hours after entry into the vascular system of the plant. Further observation indicates that the toxin-induced interference with the integrity of the cell may be in immunological responses of the plants, when NLP contacts with potential defense of the plant several times, it reaches to its peak [29], [27]. NLP induces several genes that are often associated with different stress- defense responses [12], [6], [29], But as noted NLPs are just effective on dicot plants. The lack of effect on the monocot plants identified that NLPs is not recognized by the immune system of the monocot plants, in other words, there is a receptor protein in the dicot plants that is not exist in the monocot plants and therefore they are not sensetive to any of these proteins. sSo far in studies on host range of NLP no response has been reported despite the use of monocot plants [8]. sSince NLP increases ethylene emission in tobacco leaves [9], [15], [18], It is suggested that necrosis could be an indirect effect of this hormone. However, in some plants necrosis inducing did not occur accompanied by ethylene production [9], [10]. Also NLPs disrupt the homeostasis of ions and membrane potential, leading to the activation of defense responses. Significantly NLP induces falling H^+ and Ca^{2+} and K^+ losses [15], [18]. NLP involves in stimulation of virulence -dependent genes in plant, changes in K^+ channel flow and H^+, accumulation of reactive oxygen species (ROS) and ethylene, changing in cell respiration, increased response and sensitivity to local death [15], [18], [21]. Due to the absence of stomata in the stems and roots, NLPs are just effective on leaves. In studies to evaluate the biological activity of NLPs, five absorption methods in plants have been utilized:

1. Removing leaves from petioles and putting them in Ffungal culture filtrate
2. Spraying of Ffungal culture filtrate mid detergent on leaves
3. Fungal culture filtrate injected into leaves
4. PBI121 vectors for gene transfer by Agrobacterium injection method with agerfilteration
5. transfering of *nep1* gene into a suitable expression vector, such as E.coli for obtaining more protein
   And then extract it and evaluate its effect in different ways such as spraying and etc on the desired plant
6. Hanging drop method

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Each of the above methods has different benefits; Depending on the purpose of the assay one of these methods can be selected. For example, as in the experiments of Jennings in year 2000, different ways were assayed on the leaf, including spray of the protein, absorption through the leaf petiole and injected into the leaf, resulting in even more destructive effect than other about spraying method Aand even using the spray several times for obtaining further damage has been recommended [18], [10]. Also because the ease use of this method as a biological herbicide and increased treatment areas is recommended. In the difference between extracting protein from producing microorganisms or the expression vector, the second method is recommended because of the increased production of these proteins and ensure that the desired protein is the only factor (and not other metabolites produced by the microorganism) that effects on the plant [3].

V. Effective Factors on performance of NLPs:

A. *Temperature:*

Previous research indicates that NLP activity is sensitive to temperature and less necrosis ability after exposure to temperatures of 65 ° C for 15 min [8], [18]. The sensitive biological activity of NLP to heat is so that heating this protein for 10 min at 95 ° C shows no measurable activity. However, boiling at 95 ° C for 1 hour or more, causes the protein break down and small molecular weight compounds are made that are observed in SDS-PAGE, and biological activity disappears [10], [14]. Also NLPs are stable for 38 days at 4 ° C and 28 days at 20 ° C [8], [25]

B. *The difference in the necrosis ability of NLPs:*

NLP producing Microbial Rresources, has different ability with each other, and not all proteins in a functional level [35], [15] for example, BcNEP1 from *Botrytis cinerae* is 5-10 times more potent than this protein in *Fusarium oxyporum* and therefore related symptoms are more severe [35].

C. *The effect of NLP concentration on its effectiveness:*

In several studies the effects of increasing the concentration of NLP has evaluated and it has been shown that this increasing is more destructive [18] effective concentration has a critical threshold and more and better effect cannot be seen beyond it. Also the effects of this protein on various plants are different, so that harmful effects on some plants are stronger [21].

VI. Influencing Factors on increasing the effect of NLPs:

1. In different experiments indicated that factors such as detergent enhances the effect of these proteins [18], [21], [1] so by creating turbulence in the cell membrane results in better penetration of this protein and its effects have been increased.
2. Jennings also tested the effect of ethylene pretreatment of weed leaves prior to treating with NLP, the level of induced ethylene production was increased approximately threefold in this expriment [18].
3. Moreover, several tests on the composition of the protein by fungi or bacteria are used. For example Gronwald et al [2004] examined the effects of purified NLP from its producing fungi and Pseudolonas syringae on 3 weeds of Ambrosia artemisiifolia, Cirsium arvense and Taraxacum officinale [17]. In this study, it was presented that the combination of CFU 10 from Pseudolonas syringae bacteria and 5 µg / ml of purified protein NLP caused 60 to 80 percent necrosis in leaves of these three weeds [17].
4. In another study by Bailey and colleagues in 2000 for the biological control of Opium poppy, fungal spores of Pleospora papaveracea with purified NLP from its producing fungal were used. In this study was cleared that combined use of fungal spores along 10 µg / ml of purified NLP will increase the control of Opium poppy [7].
5. In addition to the direct use of NLP, gene transfering into fungi lacking this protein was used in weed control. For example, transfering NLP gene to Colletorrichum coccodes for biological control of velvetleaf weeds, increased its pathogenicity ninefold [15]
6. Using NLP proteins from microorganisms that may be more powerful, as in the case mentioned about Botritys [35] Also using of microorganisms that have multi-gene families of NLP and assessment of their power is recommended.

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7. combining of NLP with enzymes: Thomas et al [1998] in evaluating the Effect of Fusarium oxyporum in order to control of parasitic broomrape (Orobanche Cumana) found that When seeds are treated by Fusarium cells, compounds such as pectin methyl esterase and trans Almynaz pectin is secreted by the fungi, which can cause wall pectin to dissolve and facilitate the penetration of fungi to the cell walls of the seeds [34].

VII. CONCLUSION

Obviously the use of chemical agents to control weeds is dramatically dangerous because of their environmental damage, destructive effect on human and animals, causing resistant plants. therefore seeking for safer controlling ways are required. Among the controlling ways biological control because of what mentiond above are more economic and safer. Phytotoxins are one of the bioherbicides that are produced by different microorganisms and have different size and chemical structure. Among peptid phytotoxins, NLPs are an good option and can be possible alternative of chemical herbicide in the field of monocot cultures due to their selective effect just on dicot plants, rapid disintegration and not leaving rasidues in environment and therefore having no pollution effect on environment, human and animals, affecting in short time. In order to identifying this protein from other microorganisms, maybe strong proteins could find and so, no other contributing factors on its effect is required which could be more economic.

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