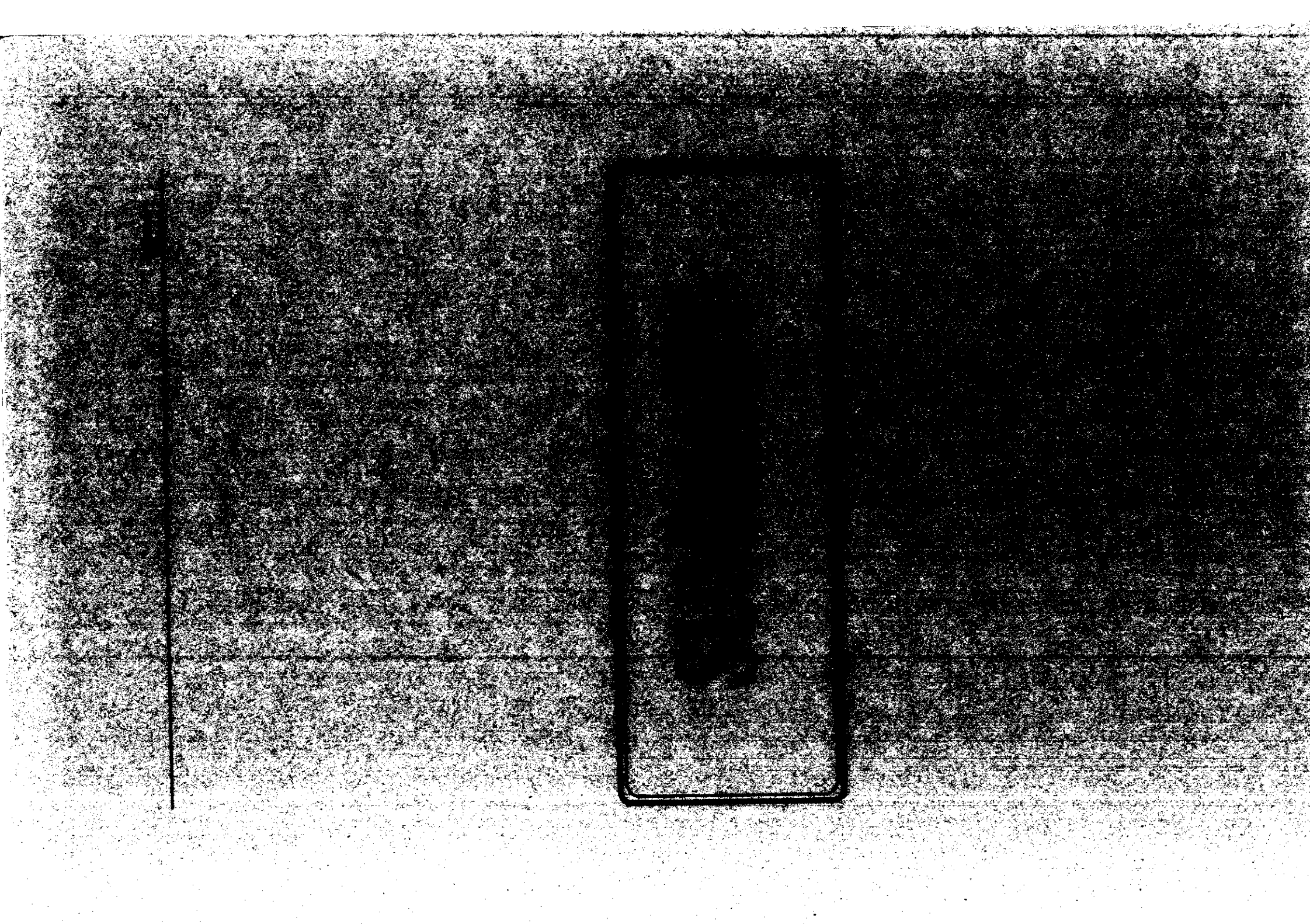


6. SUMMARY

The responses of soybean cv. Crowford and sunflower cv. Miak, susceptible to infection by the charcoal rot pathogen *M. phaseolina* and the Fusarium root rot pathogenic fungus *F. solani* f.sp. *phaseoli*, in pot studies in the greenhouse were determined. Diseases symptoms at various stages of plant development were evaluated using morphological and histological approaches. Hence, the individual pathogenic characteristics of these two fungi were firstly demonstrated on the soybean and the sunflower plants using excised organs (hypocotyls) of the plants under environmental controlled conditions.

Secondly, the pathogenicity was also employed to the seeds which were infected separately with each of the pathogens of concern, by applying its inoculum incorporation with the soil, under greenhouse condition. That methodology is important in demonstrating and clarifying pathogenicity in particular at the various stages of plant development. Both fungi caused a diversity of symptoms on both tested plants. Both fungi were considered a potential causal agents of blight as well as root and foot-rot of the infected plants, whether at the seedling stage, the vegetation stage or even at older stages (flowering and fruiting).

The pathogenic types of both fungi demonstrated marked effects on the health and physiology of the plants, wilt was also one of the most common among the other symptoms. A comparison of soybean and sunflower plants indicated that both fungi, separately, have marked effects on the health and physiology of the plants resulting in losses in seed yield.

Definitive histological studies of infection characteristics of these fungi by light microscopy are lacking in this study.

The bacterial antagonist *B. subtilis* and *T. harzianum* were all tested under greenhouse conditions. The other biocontrol agents used included the two selected isolates of

streptomyces, isolate No. 112 and isolate No. 729 which resembled the species *S. roseodiasticus* and *olivaceiscleroticus*, respectively. These were effective as the fungal biocontrol agents. The isolates collection isolated in this study was described as new. The isolation and characterization of the two isolates of *streptomyces* spp. used were achieved for the first time in this study.

Control efficiency of the antagonistic effectiveness of the tested biocontrol(s) varied slightly with all of the microorganisms used and even between the two isolates of Streptomyces . Slight differences in reducing diseases severity and rating among them was demonstrated in this study, i.e., induced disease control by close levels were achieved against the charcoal root rot of soybean and sunflower and also against the Fusarium root rot of both plants. The latter disease was also considerably decreased compared with controls. The reduction was followed by a moderate yield increase. Differences between biocontrol treatments were not significant. Moreover, all treatments in soybean and sunflower resulted in an earlier development of pods of soybean and heads of sunflower. Decreasing levels of susceptibility, in cultivar of soybean and sunflower plants normally susceptible to *M. phaseolina* and *F. solani* f.sp. *phaseoli* ,with each of the biocontrol used was apparent. Disease severity in either cv. Crowford of soybean or cv. Miak of sunflower resulted in limited to extensive pale flecking damage to the root and brownish necrotic hypocotyl lesions in addition to a limited type of symptoms on the leaves which was also apparent. At the seedling stage, in particular, a pale rotting of primary root was evident which slightly progressed into the hypocotyl. Only with Macrophomina pathogen that the rotting was restricted to the lower hypocotyl and primary root. At later stages of plant development, the normal susceptible diseases rating caused by the two pathogens was apparently altered. Plant growth promotion in soybean and sunflower were observed. High yield levels was evident based on the various plant growth parameters which have been observed. It was demonstrated that this phenomenon was accompanied with decreasing disease severity and rating in all of the Macrophomina or Fusarium - soybean or sunflower interaction systems which were involved: treated plants, biocontrol-infected plants, non treated (susceptible) infected plants, and the controls (biocontrol non treated- non infected plants).

The potential biocontrol agents have been applied separately for the first time to infested soil with the plant pathogens in pot studies under greenhouse condition. Dox liquid culture preparation of *T. harzianum* or the preparations of the other tested biocontrol were coated separately to the seeds of susceptible cultivar of soybean (cv. Crowford) as well as susceptible cultivar of sunflower (cv. Miak). When these coated seeds were infested in the soil (under greenhouse condition), which was incorporated with the appropriate inoculum of the tested target pathogen(s) via simultaneous applications of both of the biocontrol and the target pathogen, it led to reduced diseases severity.

This is the first study to demonstrate the effectiveness of all the three adverse group of micro-organisms tested as a biocontrol agents against *M. phaseolina* and *F. solani* f.sp. *phaseoli* in both soybean and sunflower under greenhouse condition. The three diverse group of micro-organisms which were used as a biocontrol agents were antagonistic to the pathogenic fungi of the two plants (soybean cv. Crowford and sunflower cv. Miak).

Preliminary screening of activities towards the tested pathogens *in vitro* (petri dishes) trials revealed inhibition to both microsclerotia production and growth of *M. phaseolina* as well as the growth of *F. solani* f.sp. *phaseoli*. More importantly the isolate (No 112) was demonstrated to be highly effective "efficient" among the biocontrol agents used in this study, i.e., *B. subtilis*, and the other fungal tested antagonist (*T. harzianum*) in controlling the diseases caused by the two phytopathogenic fungi *M. phaseolina* and *F. solani f.sp. phaseoli* in the target plants of this work

The germination and radical root growth assay for the phytotoxicity of the culture filtrates (CF_S) of the three different biocontrol agents were employed on both the soybean and sunflower seeds. Serial concentrations of these CF_S were used. A visible damage on both the soybean and the sunflower radicals was demonstrated. This was based on the length measurements of radicals (Root-inhibiting activity) in the CF_S treated seeds as compared with the water controls. This points to the contribution of a phytotoxic substance(s) which may

possess an anti-mycotic activity at an appropiate concentration. It is worthy noting that it was apparent from experimental obesrvations that adventitious roots were present with CF of *S. roseodiasticus* (No 112) in-particular at the very low concentration (10%). This points out to a possible involvement of a biologically active constituent, other than the toxins in these CF_S.

The mode of action of each of the biocontrol tested on plant metabolism has been investigated and conceivably speculated. The biochemical analyses conducted in the present study using the chromatographic techniques involved the TLC and HPLC. The analyses revealed the following:

Differences in morphology, TLC and HPLC patterns were found matching those differences in the pathogenicity to the pathogens used, the responses to biocontrol agents(s) alone, and the responses to the pathogens with biocontrol(s).

TLC analyses strongly indicated that there is a link between pathogenicity of both of the two tested pathogens on sunflower (organs) and induced isoflavonoid metabolism content of these organs as result of coating its seeds with the various biocontrol agents and infested them simultaneously in the pathogen(s) incorporated soil, under greenhouse conditions.

The various interaction systems extracts demonstrated that several of the metabolites detected are conjugates of simple flavonoid and isoflavonoids aglycones (that served as phytoalexins) of known broad range of toxicity that most possibly helped to contain the development of the pathogen(s) of concern in the soybean plants.

Isolation of aromatic compounds was essentially performed as described by researchers working with various aspects of soybean-pathogen(s) interaction systems. However, the gradient elution of the HPLC separation technique as conducted by one of those researchers was employed in this study. This procedure was adapted for profiling such aromatic compounds.

The responses of the two tested organs involved multiple forms of a family of compounds rather than a single compound.

The HPLC profiles protocol of these several aromatic compounds proved to be of value in the clinical diagnosis of diseased plant tissues and the specific disease pathogen as well as in showing the efficiency of the biocontrol agent in controlling the target disease.

The presence of these aromatic compounds, whether constitutive or inducible, was demonstrated in the susceptible interaction systems which had become biocontrolled resistant.

The role that this metabolism may play in infected tissues and in biocontrol protected tissue was also discussed in soybean while in the sunflower plant the knowledge about this role is still incomplete.

The detectable metabolites and their levels and the proportions of some of these metabolites (known ones) in all the systems studied have indicated that the plant pathogenic fungi tested may actively metabolized some of the various soybean isoflavonoids.

The distributions of specific isoflavonoids phytoalexins in these various soybean plant-pathogen(s)/biocontrol(s) tissues were also investigated. This pointed out to conceivably speculate that the production of several specific metabolites may be involved in the resulting biocontrol interaction systems rather than the overall production process. Unidentified isoflavonoid compounds were also investigated as being phytoalexins. The HPLC analyses revealed the presence of highly prominent compounds at R_{ts} 18.0-18.5, 23.0-23.9, and 22.5-22.9 min. In addition to other compounds of lesser levels than those of the highly prominent ones at R_{ts} 13.0-13.5, 17.5-17.9, 19.0-19.5, 20.5, 20.9, 21.0-21.5, 24.5-24.9, 26.5-26.9 and 27.0-27.5 min. The proposed role of these unknown compounds as well as the other identified ones in the biocontrol of the diseases concern was also clarified.

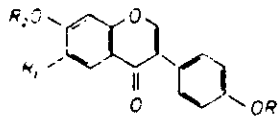
Variation in level between the detectable aromatic secondary metabolites, which included the isoflavonoids, isoflavonoids glucosides and its conjugates phytoalexins in seedling of 18 day-old soybean plants of varying organs (hypocotyls and leaves) were detected . These phytoalexins were daidzein, genistein and glyceollin, and they were either constitutive and/or inducible ones and were of phenolic nature.

Increased levels of pools of some of these metabolites which are phenolic precursors that were present in the analysed tissue of concern was investigated.

The target metabolic processes in the soybean, particularly the phenylpropanoid pathway which resulted in the formation of the aromatic phenolic products, were only demonstrated as mentioned above. These processes were discussed with references to the studied plant-pathogen(s) systems as well as with those of other researchers involving the same host but with other non-related pathogen, i.e., *Phytophthora megasperma.* f.sp. *glycina* (normal susceptible and resistant inter action systems).

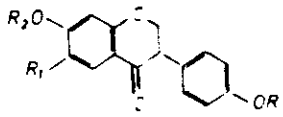
A stereoscaning electron microscopic comparison of the responses of the inoculated sites of excised hypocotyl tissue of the susceptible cultivar of soybean cv. Crowford to inoculation, firstly, by each of the three biocontrol agents used and 24 h later each of the two pathogens of concern was applied.

The pattern of responses of the pathogenic fungus and the biocontrol in addition to the host response by S.E.M was clarified during the early stages of infection. This has led to clarfying the nature of the antagonistic relationships involved in the examined interaction systems, therefore, clearly establishing the mechanism(s) of biocontrol resistance by the S.E.M.



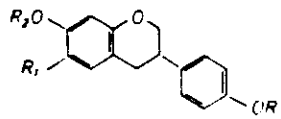
Isoflavones

1 Gyorgy - isoflavone
2 Daidzein
3 Glycitein
4 Formononetin



Isoflavanones

5, 6, 7, 8



Isoflavans

9, 10, 11, 12

1, 5, 9 = (R, R_2 = H, R_1 = CH)
2, 6, 10 = (R, R_1, R_2 = H)
3, 7, 11 = (R, R_2 = H, R_1 = OCH_3)
4, 8, 12 = (R = CH_3, H, R_2 = H)

Fig. 49 Chemical structure of isoflavones and their reduced derivatives.

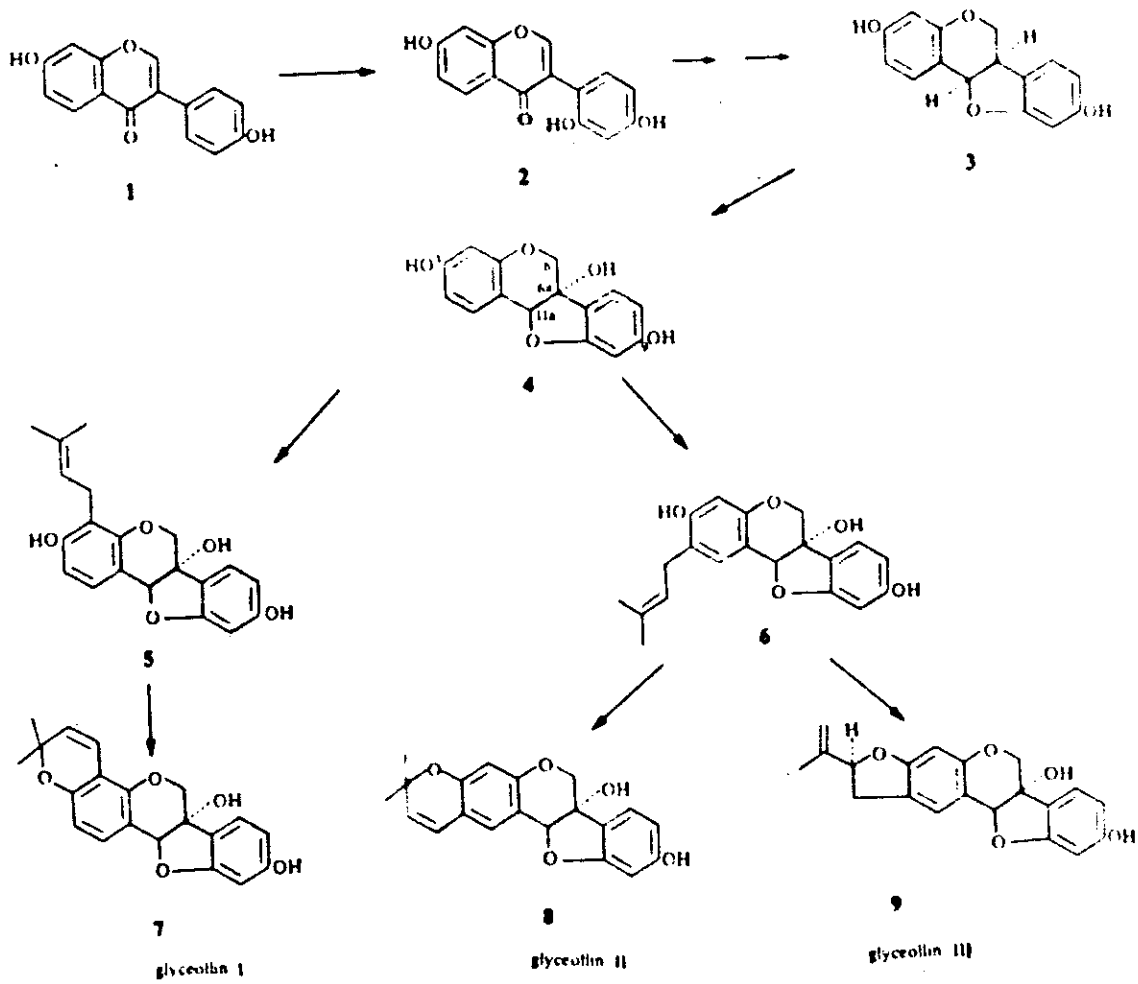


Fig. 50 Scheme for biosynthesis of glyceollins I, II and III in glycine max.