BANDING PATTERN OF ACIDIC AND BASIC PROTEINS FROM CHICKPEA (*Cicer arietinum* L) ROOT TISSUE INFECTED WITH FUSARIUM WILT (*Fusarium oxysporium* f. *spp. ciceri*)

***RATHOD, P. J. AND VAKHARIYA, D. N.**

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF AGRICULTURE
JUNAGADH AGRICULTURAL UNIVERSITY
JUNAGADH-362001.(GUJARAT) INDIA

EMAIL: dr_pankajkumar@hotmail.com

ABSTRACT

An experiment was conducted during 2006-2008 at Junagadh to study the change in acidic and basic protein at different stages of infection in chickpea grown in normal and sick plot. Results indicated that Native-PAGE banding pattern of basic protein at pre-infectional stage (S_1) root tissue of all cultivars showed total five protein bands of basic proteins. Out of five, four bands were clearly visible in JG-62 and GG-4. Same cultivars were grown in normal soil had seven visible protein bands. Native-PAGE banding pattern of acidic protein at pre-infectional stage showed 11 protein bands in all the cultivars grown either in normal plot or sick plots. At post-infectional stage (S_3), there were no differences in protein banding pattern either in acidic or basic protein or plants grown in normal or sick plot. SDS-PAGE banding pattern of basic protein showed cultivar differences at pre-infectional stage. Total 12 proteins bands were recorded in JG-62 and GG-4 grown in normal soil, while JCP-27, GG-1, GG-2 and WR-315 showed the presence of total 14 bands. Whereas WR-315, JCP-27, GG-1 and GG-2 possessed 14 protein bands at infectional stage. At post infectional stage (S_3) or with the progress of growth, the number of protein bands remain same either they were grown in normal plot or sick plot. SDS PAGE banding pattern of acidic protein at post-infectional stage resulted two extra acidic protein bands in cultivars JG-62, which clearly indicates that new acidic proteins were formed due to fungal infection.

KEY WORDS: Acidic, Basic protein, Chickpea, Native PAGE, SDS

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop of the world which provides high quality protein especially in a vegetarian diet. The crop is affected by various abiotic and biotic stresses. A number of pathogens affecting chickpea have shown a threefold increase during last 15 years (Nene *et al.*, 1996). The main fungi that affect chickpea are *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *ciceri* (Padwick) Matuo & K. Sato, causing the plant to wilt. Wilt of chickpea, caused by *Fusarium oxysporum* f. sp. *ciceri* is a major limiting factor of chickpea production in the Mediterranean Basin and the Indian Sub-continent (Jalali and Chand, 1992). It is prime important to understand the host pathogen interaction in chickpea plant. accumulation of PR-proteins and cell wall

enforcement by oxidative cross-linking of structural proteins and the formation of papillae have been documented in the interaction between *F. graminearum* and wheat (Pritsch *et al*., 2000; Pritsch *et al*., 2001; El-gendy *et al*., 2001; Kang and Buchenauer, 2003), therefore, the experiment was carried out on changes protein profile in chickpea roots grown in normal and inoculated soil with *Fusarium oxysporum* f. sp. *ciceri* at different stages of development. Hypothesis based on that there may be role of novel protein on host and pathogen interaction.

MATERIALS AND METHODS

An experiment was conducted at Junagadh Agricultural University, Junagadh to identify the acidic and basic protein banding pattern changes in chickpea in response *Fusarium* wilt (*Fusarium oxysporum* f.sp *ciceri*). Chickpea cultivars *viz*., V_1 (WR-315 Highly Resistant); V_2 (JCP-27 Resistant) ; V_3 (GG-1 Tolerant) ; V_4 (GG-2 Tolerant) ; V_5 (GG-4: Susceptible) and V_6 (JG-62: Highly susceptible) were grown under field condition in two plots. One plot was normal plot without diseased, while other was kept free for infection of wilt disease in chickpea plants in sick plot that is maintained since 20 years for *F. oxysporium f. sp. ciceri*. Root tissues were collected at pre-infectional (12 Days after sowing), infectional (21 days after sowing) and post-infectional (26 Days after sowing) stage from both the plots. Sample was preserve in -80^{0}C until analysis is over.

Protein banding pattern of pathogenesis related (PR) proteins on PAGE

Proteins were extracted with 0.1M (pH 7.2) Na- phosphate buffer. 500 mg of root tissues from each sample was separately homogenized in O.5 ml 0.1 M of sodium phosphate buffer pH 7.2. Homogenate was centrifuged at 10,000 rpm at –4^{0}C in refrigerated centrifuge for 10 minutes. Clear supernatant obtained was used for PAGE. Ten per cent polyacrylamide gel was used for the separation of proteins as per the method described by Laemmli (1970).

Basic proteins extraction

Extraction of basic PR proteins from chickpea root tissues was done using 0.1M acetate buffer pH 4.8. Basic proteins were studied through native and SDS PAGE. Tissues analyzed and procedure adopted for PAGE was same as described above for acidic protein extraction.

Sample preparation for Native PAGE

50 µl of protein extract having 50 µg of protein was mixed with 2 µl 0.5 per cent bromophenol solution and 20 µg sucrose. The mixture was applied to each well. Electrode buffer pH 8.3 was freshly prepared and used. Electrophoretic run was started at 20 mA constant current and was completed after 2.5 h. Gel was carefully removed and was stained for 2 h in coomassiae brilliant blue dye. Destaining of the gel was done by dipping the gel in destaining solution and proteins separated were observed.

Preparation of Polyacrylamide Gel

Polyacrylamide gel was prepared as per the method described by Laemmli (1970) and Davis (1964). Running gel solution of 10 per cent prepared as described above was poured in gel casting unit (Biotech model). Saturated butanol solution was layered over the poured gel to prevent curve formation. Gel was cast in 30 minutes and saturated butanol was removed from the cast gel. Four per cent stacking gel solution prepared as described above was poured on the cast running gel. As the stacking gel cast quickly, comb for 12 wells was kept immediately after the pouring the stacking gel solution. The sample of protein prepared was applied to each well. Electrode buffer was freshly prepared and used. Electrophoretic run was started at 20 mA constant current and was stained for 2 h in staining solution. Destaining of the gel was done with destaining solution. Protein bands observed and their relative mobility value (Rm) values were recorded.

SDS-Polyacrylamide Gel Electrophoresis

To study the protein banding pattern on SDS PAGE following modification were made in above referred native PAGE preparation. 15 mM phenylmethyl sulfonyl fluride were added in 0.1M PH 4.8 acetate buffer. During gel casting SDS were added in above referred running gel (0.3ml SDS/30ml) stacking gel (0.1ml SDS/10ml) preparation. Electrode buffer (pH 8.3) was mixed with 10 ml of 10 % SDS/litre .

Sample preparation for SDS PAGE

1 ml crude protein extract was mixed with 1 ml sample buffer and boiled for 3 minutes. After cooling 50 ul (100 ug protein) was applied for electrophoresis. After electrophoresis run, gel as immersed in 10 per cent acetic acid solution for 10 minutes to remove excess SDS. Gel was then stained in dye as described earlier and after destaining it was scanned under gel scanner.

Preparation of Polyacrylamide Gel

Polyacrylamide gel was prepared as per the method described by the Laemmli(1970) and Devis(1964). Running gel solution of required percentage prepared as described above. To study the SDS proteins banding pattern of phosphate buffer extracted proteins following modification were made in methods. During protein extraction 0.1 ml 10 percent SDS was added. 1ml of protein extracted solution was mixed with 1ml 10 per cent SDS was added. 1 ml protein extracted solution mixed with 1ml buffer and boiled for 1 minute. After cooling 50ul (25ug protein) was applied for electrophoresis. Sample buffer was prepared mixing 1) 0.5ml tris HCl buffer pH 6.8, 2)0.05g SDS, 3) 0.5 g sucrose, 4) 0.025 ml β-Mercaptoethanol, 5) 0.1 ml 0.5 percent bromophenol blue and final volume was made to 1 ml with GDW. 10 per cent SDS was also used in preparation of the following solution; running gel (0.3ml SDS/30ml), stacking gel (0.1ml SDS/10ml) and running buffer solution (10ml/litre). After electrophoretic run, gel was immersed in 10 percent acetic acid solution for 10 minutes to remove excess SDS.

RESULTS AND DISCUSSION

Native-PAGE banding pattern of basic protein at different stages

Banding pattern of basic proteins extracted from chickpea roots of all the cultivars grown in either normal plot or sick plot obtained from native PAGE are depicted in plate 1 and Figure 1. At pre- infectional stage (S_1), root tissue of all cultivars showed total five protein bands of basic proteins (Plate 1 and Figure 1). Out of five, four bands were clearly visible in JG-62, GG-4, while one band was very faint (Rm 0.16). Cultivar GG-2 (V_3) showed similar banding pattern but the intensity of bands were more as compared with the other cultivars. Resistant / tolerant cultivars WR-315, JCP-27, GG-1 and GG-2 possessed all the protein bands but they were very faint. Root tissues of chickpea grown in sick plot showed six protein bands in WR-315 and JCP-27, the intensity of protein band were more in cultivars JCP-27, GG-4 and JG-62. However, the cultivar GG-4 and JG-62 consist of total 8 bands at pre infectional stage (S_1). Thus, the root tissues from cv. GG-4 and JG-62 resulted with two extra bands having Rm value of 0.87 and 0.93.

Basic protein extracted from root tissue at infectional/growth stages (S_2) were subjected to native PAGE and protein banding patterns are presented in plate 1. As seen in the plate 1, root tissues of JG-62 and GG-4 grown in normal soil consist of seven visible protein bands of Rm 0.16 0.24, 0.41, 0.43, 0.47, 0.60 and 0.87. Out of which one band was faint having Rm 0.16. Cultivar WR-315 grown in normal soil showed the presence of 8 bands. Chickpea plants grown in sick plot resulted in seven protein bands of basic proteins in cultivar JG-62 and GG-4 and the intensity of protein bands was lower than the rest of the cultivars.

At post-infectional stage (S_3) or with the progress of growth, the number of

protein bands remain same either the plants were grown in normal plot or sick plots. However, there were little differences in their Rm values.

Native-PAGE banding pattern of acidic protein

Chickpea cultivars grown in normal plot or sick plot were subjected to extraction of acidic proteins from root tissues using phosphate buffer pH 7.2, the acid proteins were subjected to native PAGE and banding pattern of acidic proteins are shown in Plate 1 and Figure1. Total 11 protein bands were seen in all the cultivars grown either in normal plot or sick plots having Rm value of 0.20, 0.23, 0.28, 0.33, 0.41, 0.49, 0.55, 0.61, 0.67, 0.72 and 0.80 at pre-infectional stage. Though there were differences in the intensity of protein bands. Overall results indicated that the protein banding pattern in all the cultivars were identical at pre-infectional stage. With the advancement of growth or progress of disease (S_2), plants grown in normal plot (Plate 1 and Figure 1), resistant cultivars (WR-315 and JCP-27) or tolerant cultivars (GG-1 and GG-2) showed the presence of 8 protein bands on native-PAGE, while susceptible cultivars JG-62 and GG-4 resulted seven clear visible bands. However, the intensity of protein bands was more in JG-62 and GG-4. Root tissues obtained from the plants grown in sick soil had one less band having Rm value of 0.67 in JG-62 and GG-4. At post-infectional stage (S_3) there were no differences in protein banding pattern either plants grown in normal or sick plot. However, the intensity of the bands varied from cultivar to cultivar.

SDS PAGE banding pattern of basic protein

Banding pattern of basic proteins extracted from chickpea roots of all the cultivars grown in either normal plot or sick plot obtained from SDS PAGE are depicted in plate 1 and Figure 1. As seen in plates root tissues of JG-62 and GG-4 grown in normal soil consist of total 12 proteins bands and 9 protein bands were clearly visible, while three were very faint. However, protein banding pattern of Cv. JCP-27, GG-1, GG-2, and WR-315 revealed presence of total 14 bands and 12 bands were clearly visible. Cultivars JCP-27, GG-1, GG-2 and WR-315 consist of two additional bands having Rm value of 0.71 and 0.74 as compared to JG-62 and GG-4. Resistant cultivar JCP-27 showed two additional bands as compared to JG-62 and GG-4 grown in sick plot. However, the intensity was more pronounced in JG-62 and GG-4 grown in sick plot. Protein banding pattern of root tissues of resistant cultivar WR 315 grown in sick plot showed the presence of total 13 bands, while cultivar GG-1 and GG-2 resulted with 14 bands. Separated proteins on SDS PAGE showed lower intensity of bands in JG-62 and GG-4. At pre-infectional stage (S_1), root tissues of all cultivars showed total 14 protein bands of basic proteins. Of 14 bands, 9 bands of basic proteins were clearly visible in JG-62 and GG-4, while two bands were very dark in all the cultivars at all stages from S_1 to S_3 and at same Rm value. All cultivars showed similar banding pattern but the intensity of bands were more as compared with the other cultivars. Resistant and tolerant cultivars WR-315, JCP-27, GG-1 and GG-2 grown in normal plot showed higher intensity of protein bands at all stages exceptionally good variation among the band width and migration of proteins of sick plot grown same cultivars. Root tissue of chickpea cultivars GG-2 (V_4), GG-4 (V_5) and JG-62 (V_6) having same banding pattern as in normal and sick plot but WR-315 (V_1) and GG-1 (V_3) showed one missing band as compared to cultivars grown in normal plot at S_1 stage. However, the cultivar differences were observed but not major differences in basic proteins at pre-infectional stage (S_1). Thus, the root tissues from JG-62 (V_6) possessed only 12 bands in both the plots at this stage. In general, basic protein extracted from root tissue at infectional/growth stages

(S_2) were subjected to SDS and protein banding patterns are presented in plate 1. Root tissues of JG-62 and GG-4 grown in normal soil showed 12 clearly visible protein bands out of which one band was faint. Cultivar WR-315 grown in normal plot showed the presence of 13 bands. Chickpea plants grown in sick plot resulted 13 protein bands of basic proteins in cultivar JG-62 and GG-4 and the intensity of protein bands was lower than the rest of the cultivars. Cultivar WR-315, JCP-27, GG-1 and GG-2 contained 14 protein bands. Thus, these cultivars had one additional band compared to JG-62 and GG-4. At post-infectional stage (S_3) or with the progress of growth, the number of protein bands remain same either they were grown in normal plot or sick plot. However, the differences in their Rm value were observed as seen in plates.

SDS PAGE banding pattern of acidic protein

Banding pattern of acidic proteins extracted from chickpea roots of all the cultivars grown in either normal plot or sick plot obtained from SDS PAGE are depicted in plate 1 and Figure 1. At pre-infectional stage both the plot grown cultivars differences were remain identical but band intensity showed more darker than the normal plot grown cultivars having Rm value of 0.16, 0.24, 0.41, 0.43, 0.47, 0.60, 0.87 and 0.93. In case of infectional stage (S_2), cultivar differences were not observed as seen in sick plot of S_1 stage. Only five major bands were observed with the similar Rm values in all the cultivars. In case of post-infectional stage, two acidic protein bands were recorded in JG-62 and it clearly indicated that new acidic proteins were formed due to fungal infection with the Rm value 0.68 and 0.72. Overall results obtained from acidic and basic protein of native as well as SDS PAGE are supported with Shukla (2001). They studied chickpea wilt infection at different stages of infection in root tissues only with cultivar JG-62 and JCP-27. Prasad *et al*. (2003) studied on the genetic variability among the resistant and susceptible cultivars by root dip inoculation with *Fusarium udum* [*Gibberella indica*] and their electrophoresis showed induction of two specific bands of root peroxidase (Em=0.24 and 0.55) on native-PAGE in ICP 8863 after inoculation. The results are also in agreement with the Raskar *et al*. (2005). They analyzed PRPs on Native as well as SDS-PAGE at 25 DAS for *Fusarium* wilt in chickpea root tissues of resistant and susceptible cultivars grown separately in sterilized (control) and wilt sick soil and revealed that some novel PRPs synthesized in resistant cultivars mainly in root portion of chickpea. In root portions the syntheses of acidic PRs of the size of 23.7 kD on native-PAGE and 23.0, 33.5 and 66.0 kD on SDS-PAGE. Saikia *et al*. (2006) observed acidic PR protein of 22.0 kD size in root portion of resistant cultivar of chickpea.

CONCLUSION

SDS PAGE banding pattern of acidic protein at post-infectional stage resulted two extra acidic protein bands in cultivars JG-62, which clearly indicates that new acidic proteins were formed due to fungal infection.

REFERENCES

Davis, B. J. (1964). Disk electrophoresis II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci*., **121**: 404-427.

El-Gendy, W.; Brownleader, M. D.; Ismail, H.; Clarke, P. J.; Gilbert, J.; El-Bordiny, F.; Trevan, M.; Hopkins, J.; Naldrett, M. and Jackson, P. (2001). Rapid deposition of wheat cell wall structural proteins in response to *Fusarium*-derived elicitors. *J. Exptl. Bot.*, **54**: 85-90.

Jalali, B. L. and Chand, H. (1992). Chickpea wilt. In: Singh, U. S.; Mukhopadhayay, A. N.; Kumar, J. and Chaube, H. S. (Eds) Plant Diseases of International Importance, Vol. 1: Diseases of Cereals and

Pulses. Prentice Hall, Englewood CliVs, New York, pp. 429-444.

Kang, Z. and Buchenauer, H. (2003). Immunocytochemical localization of cell wall bound thionins and hydroxyproline-rich glycoproteins in *Fusarium culmorum*-infected wheat spikes. *J. Phytopatho.*, **151**: 120-129.

Laemmli, U. K. (1970). Cleavage of structural proteins T4. *Nature*, 227: 680-685.

Nene, Y. L.; Sheila, V. K and Sharma, S. B. A (1996). A world list of chickpea and pigeonpea pathogens, 5th Edition, ICRISAT, Patencheru, 1996, p. 27.

Prasad, P.; Reddy, N. P. E.; Anandam, R. J and Reddy, G. L. (2003). Isozymes variability among *Fusarium udum* resistant cultivars of pigeonpea (*Cajanus cajan* (L.) (Millsp)). *Acta Physiologiae Plantarum*. **25**: 221-228.

Pritsch, C.; Muehlbauer, G. J.; Bushnell, W. R.; Somers, D. A. and Vance, C. P. (2000). Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Mol. Plant Microbe. Interaction,* **13**: 159-169.

Pritsch, C.; Vance, C. P.; Bushnell, W. R.; Somers, D. A.; Hohn, T. M. and Muehlbauer, G. J. (2001). Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. *Physiol. Mol. Plant Patho.*, **58**: 1-12.

Raskar, S. T.; kale, A. A. and Munjal, S. V. 2005. Identification of pathogenesis related proteins involved in *Fusarium* wilt resistance in chickpea cultivars. 4th International Food Legumes Research Conference, October 18-22, New Delhi. IFLR Abstract, A-693. p. 294.

Saikia, R.; Singh, B. P.; Kumar, R. and Arora, D. K. 2005. Detection of pathogenesis related proteins-chitinase and β-1,3-glucanase in induced chickpea. *Curr. Sci*., **89**: 659-663.

Shukla, Y. M (2001). Ph. D dissertation, Submitted at Gujarat Agricultural University, Sardarkrushinagar.

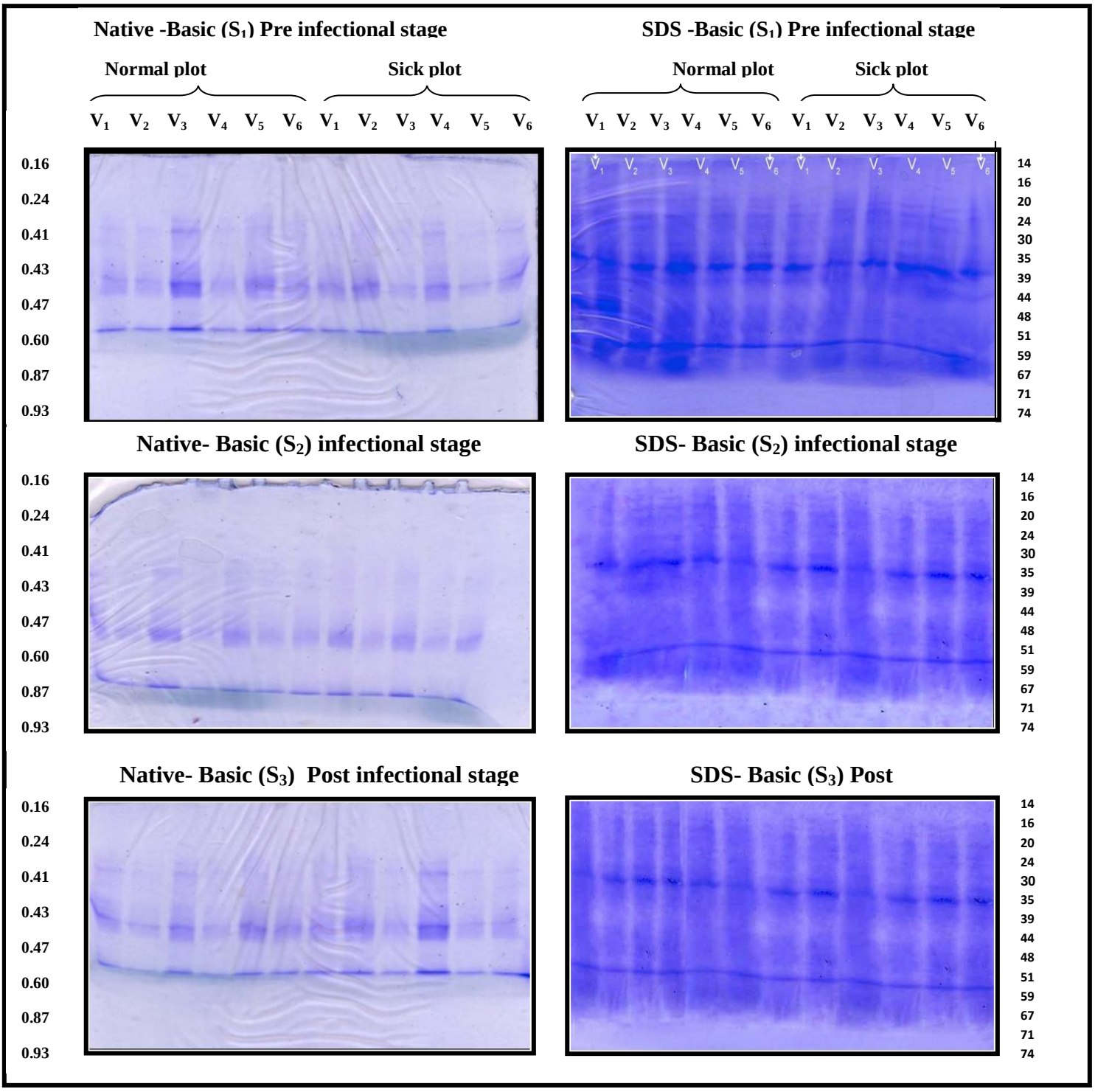

Plate 1: Native and SDS-PAGE banding pattern of basis proteins of chickpea root tissues at different stages of infection

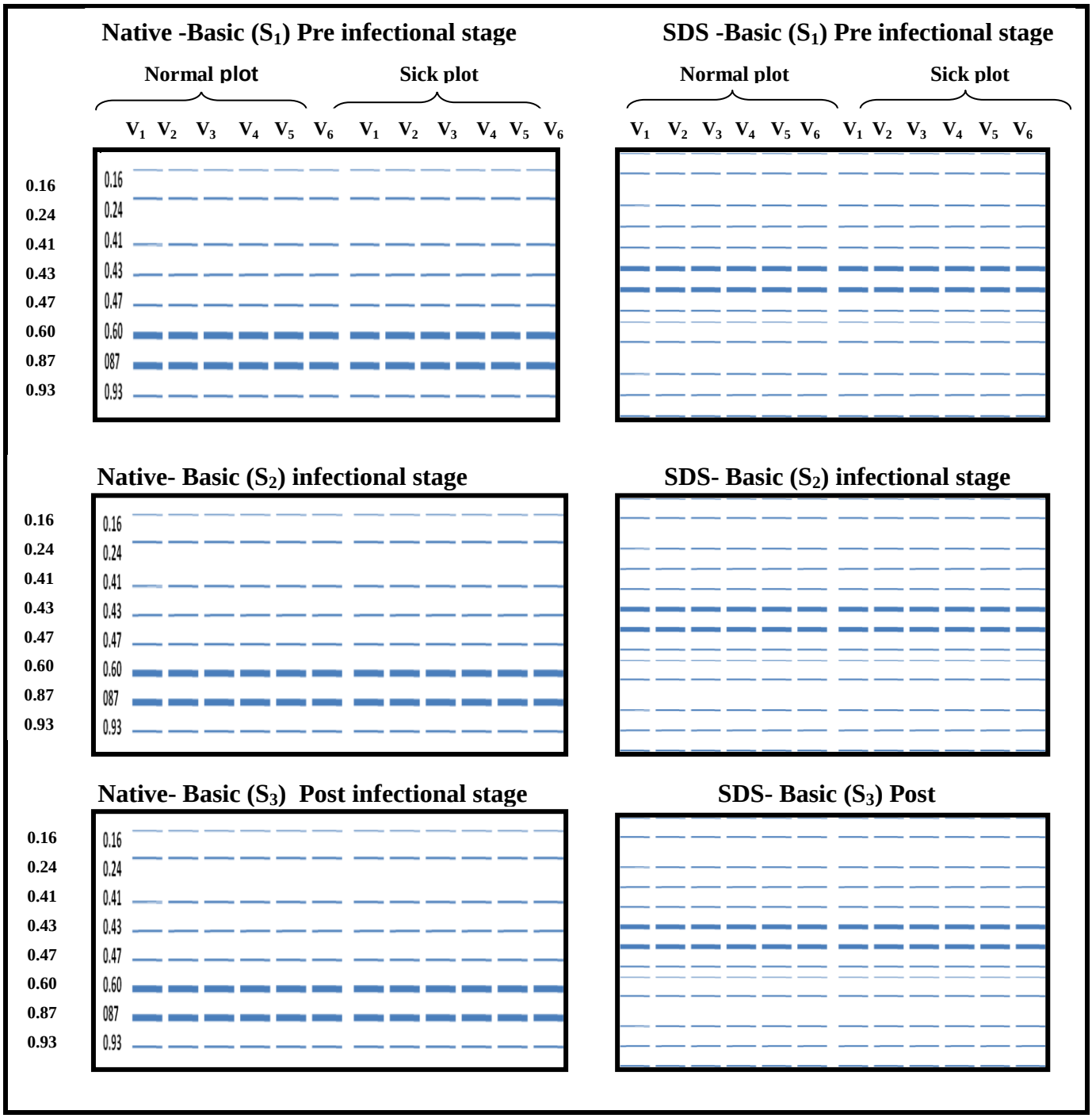

Fig. 1: Native and SDS-PAGE banding pattern of basis proteins of chickpea root tissues at different stages of infection

[MS received : December 25, 2018] *[MS accepted : December 29, 2018]*