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Influence of water stress and Rhizobial inoculation on accumulation of flavonoids and anthocyanins in selected Common bean (*Phaseolus vulgaris* L.) cultivars

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Abstract

A two season field experiment and a single season screen house experiment were conducted to assess the effect of water stress periods and rhizobial inoculation in five (5) *P. vulgaris* (L.) cultivars. The experiment consisted of 2 levels of rhizobia (with and without rhizobial inoculation), two stress levels (with and without water stress) and five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA*). Results showed that flavonoid and anthocyanins (g^{-1}.DM) concentrations were higher in non- inoculated and water stressed treatments. Varieties F8 Drought line, JESCA, and F9 Kidney Selection significantly recorded higher flavonoid and anthocyanins content in both field and screen house experiment as compared with the other tested varieties. Significant interactive effects were also observed between inoculation, water stress periods and the tested *P vulgaris* (L.) varieties.

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Introduction

Nitrogen (N) is an essential major element for growth and productivity of plants. N is a building block of proteins and important in enzyme biosynthesis and amino acids (Ayoola, 2010). Phenolic N containing compounds is extensively distributed secondary plant products and is generally derived from L-phenylalanine through nitrogen framework of cinnamate under phenyl propanoid metabolism (Razal *et al.*, 1996). Flavonoids are usually synthesized using phenylalanine which may be affected by nitrogen metabolism. Under condition of low N, the levels of Phenylalanine ammonia-lyase (PAL) activity increase hence enhances the accumulation of flavonoids (Stewart *et al.*, 2001; Mierziak *et al.*, 2014; Kondorosi *et al.*, 1995). Study by Liu *et al.* (2010) in *C. morifolium* leaves showed that flavonoid concentrations were higher under low nitrogen supply, which implies the activity of PAL (Phenylalanine ammonia-lyase) was abundant in the leaf of *C. morifolium*. It has been reported that N deficiency results in huge accumulation of secondary compounds mainly phenolics such as flavonols (Stewart *et al.* 2001) and anthocyanins (Chalker-Scott, 1999). For instance, Awad and Jager, (2002) reported a decline in the concentration of flavonoids in the skin of apple as a result of nitrogen (N) supply. Other study by Ibrahim, *et al.* (2011) on *Labisia pumila* (sub- herbaceous plant) showed a significantly less production of phenolics. It can be concluded that flavonoids metabolism in plants is highly favored in the presence of Phenylalanine ammonia-lyase (PAL) as a result of N deficiency in growth-promoting N availability such as rhizobial inoculation.

Scarcity of water is a severe environmental constraint to plant productivity as it causes a severe physiological, biochemical and molecular changes in plants (Siddiqui *et al.*, 2015). It tends to distress some crucial process in plants such as respiration, translocation, ion uptake, carbohydrates and nutrient assimilation (Farooq *et al.*, 2008). During water stress periods, higher plants are forced to produce some secondary metabolites which facilitate the plants to interact with their environment and adapt to the conditions (Ramakrishna and Ravishankar, 2011).

Secondary metabolites have been considered to play crucial roles in various biochemical processes in plants (Ramakrishna and Ravishankar, 2011; Horbowicz *et al.*, 2008). For example, phenolic compounds such as flavonoids and anthocyanins are known to play key role(s) in plant growth and development, defense of plants against insect pests and diseases, phytopathogens, signaling during nodulation (Chalker-Scott, 1999; Dixon & Steele, 1999; Ndakidemi and Dakora, 2003; Falcone Ferreyra, *et al.*, 2012). In general, among the many functions currently recognized to be done by plant flavonoids; are such as modulation of enzymatic activity, UV light protection, oxidant or free radical protection, allelopathy, insect attraction or repulsion, nectar guides, probing stimulants, viral, fungal and bacterial protection, nodulation in leguminous plants, pollen germination etc. (Ramchandra and Ravishankar, 2002).

Flavonoids and anthocyanins are reported to accumulate in plants when subjected to various stress conditions. The production of secondary metabolites in plants can be considered as a strategy of enhancing the defensive mechanism in plant when subjected to nutrient and water stress such as drought. Therefore, this study was conducted to assess the influence of water stress and rhizobial inoculation in accumulation of flavonoids and anthocyanins in selected common bean (*Phaseolus vulgaris* L.) cultivars.

Material and methods

Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520m.a.s.l. The field trial was carried out during dry season of January, to March 2014 and January, to March, 2015 while the screen house experiment was carried out from mid-January to March, 2016 under irrigation.

Experimental Design and treatment application

The experiment was designed in split, split plot with 3 replications. The plot size was 3x4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA*) were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds lines and/or varieties *KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA* were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, wooden box technique was used to establish the experiment using the protocol developed by (Agbicodo *et al.*, 2009) with some modifications. This was done by collecting the same soil used for field experiment. The common bean seeds were thoroughly mixed with *R. leguminosarum* inoculants to supply (10^9cells/g seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

Plant Harvest and Sample Preparation

Shoot plant samples from field and glasshouse experiments were collected for flavonoids and anthocyanins analysis. In the field experiment, 10 plants were randomly sampled from the middle rows of each plot while in the glasshouse experiment two plants from each pot were sampled. The shoots of the plants samples were oven dried at 60°C for 48 hours, ground into a fine powder (2 mm sieve) for flavonoids and anthocyanins analysis.

Measurement of Flavonoids (g^{-1} DM) and Anthocyanins (g^{-1} DM) levels in shoots of P. Vulgaris (L.)

Flavonoids and anthocyanins concentration in plant parts were measured by the method described by Makoi *et al.* (2010b). In this method, 0.10 g of well-ground (0.85 mm) plant material was weighed and mixed with 10mLs of acidified methanol prepared at a ratio of 79:20:1 MeOH H_2O HCl. The mixture was incubated for 72 h in darkness for auto-extraction, filtered through Whatman paper Number 2 and absorbance of the clear supernatant measured spectrometrically at 300, 530, and 657nm using acidified methanol as standard. Concentrations of flavonoids was measured using 2800 UV-Vis Spectrophotometer at 300nm and expressed as Abs g^1 DM (Mirecki and Teramura, 1984), while anthocyanins concentration in plant shoots was measured as Abs530-1/3Abs657 (Lindoo and Caldwell, 1978) and expressed as Abs g^{-1} DM.

Concentrations of flavonoids compounds were expressed as:

Flavonoids (Abs g^{-1} DM) = Abs_{300}

Anthocyanins content was calculated as described in Lindoo and Caldwell, (1978):

Anthocyanins (Abs g^{-1} DM) =Abs_{530} -1/3 Abs $_{657}$

Statistical Analysis

A 3-way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software program of 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

Results

Effect of inoculation with R. leguminosarum biovar phaseoli and stress periods on flavonoids (g^{-1}.DM) in selected P. vulgaris (L.) varieties

There were significant increases in flavonoids concentration (g^{-1} DM) in *P. vulgaris* (L.) shoots on non-inoculated treatments as compared with inoculated treatments by 18% in season one at vegetative stage and 28% in season two at flowering stage respectively (Table 1).

In screen house experiment, there was a significant increase in flavonoids (g^{-1} DM) on non-inoculated treatments as compared with inoculated treatments at vegetative stage by 3% (Table 3). Water stress significantly increased flavonoids (g^{-1} DM) content by 15% in season one at flowering stage and 30% in season two at vegetative stage (Table 1). For the screen house experiment, water stress significantly increases flavonoids (g^{-1} DM) content by 61% at flowering stage (Table 3). Significant increase in flavonoids (g^{-1} DM) content was recorded in varieties F8 Drought Line, JESCA and F9 Kidney Selection as compared with varieties KAT B9 and KAT B1 under field experiments (Table 1). Similarly, significant increase in flavonoids (g^{-1} DM) concentrations was also recorded in varieties F8 Drought Line, JESCA and F9 Kidney Selection in screen house experiments (Table 3).

Table 1. Effects of inoculation with *R. leguminosarum,* water stress and five *P. vulgaris* (L.) varieties on the accumulation of Flavonoids (g^{-1} DM) in common bean shoots for two consecutive season's field experiment.

	1st Season		2nd Season	
Growth Phases	Vegetative	Flowering	Vegetative	Flowering
Treatments inoculation				
R+	2.85±0.05b	2.91±0.07a	3.00±0.17a	2.75±0.10b
R-	3.46±0.06a	2.92±0.08a	3.08±0.18a	3.81±0.06a
Stress Levels				
S_1	3.13±0.07a	2.69±0.04b	2.51±0.17b	3.27±0.14a
S_2/S_3	3.18±0.09a	3.15±0.07a	3.57±0.12a	3.29±0.12a
Varieties				
V_1	2.97±0.12b	2.58±0.03c	2.46±0.21b	2.87±0.21b
V_2	3.06±0.11ab	2.72±0.08c	2.20±0.25b	2.98±0.20b
V_3	3.24±0.15a	2.95±0.10b	3.42±0.19a	3.58±0.19a
V_4	3.26±0.13a	3.19±0.11a	3.72±0.18a	3.62±0.17a
V_5	3.25±0.11a	3.16±0.11a	3.40±0.27a	3.34±0.17a
3-Way Anova (F-Statistics)				
Rhz	69.44***	0.01ns	0.30ns	137.22***
StrL	0.39ns	84.01***	51.14***	0.02ns
Vrty	2.58ns	22.37***	16.22***	11.31***
Rhz*StrL	9.04**	2.26ns	0.10ns	2.64ns
Rhz*Vrty	0.65ns	1.32ns	0.25ns	1.27ns
StrL*Vrty	0.20ns	4.60**	1.79ns	0.65ns
Rhz*StrL*Vrty	0.05ns	0.68ns	0.20ns	0.80ns

+R: With *R. leguminosarum*; –R: Without *R. leguminosarum;* S_1: No water stress. S_2: Water stress at Vegetative Stage. S_3: Water stress at Flowering Stage. V_1: *KAT B9*. V_2: *KAT B1*. V_3: *F9 Kidney Selection*. V_4: *F8 Drought Line*. V_5: *JESCA*. Values presented are means ± SE. **, *** = significant at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Effect of inoculation with R. leguminosarum biovar phaseoli and stress period on anthocyanins (g^{-1} DM) in selected P. vulgaris (L.) varieties

Anthocyanins (g^{-1} DM) concentration significantly increased by 71% in non-inoculated treatment in season one at flowering stage and 48% in season two at vegetative stage (Table 2). In screen house experiment, significant increase in anthocyanins (g^{-1} DM) content was 7% and 8% in non-inoculated as compared with inoculated treatment at vegetative and flowering respectively (Table 3). Water stress significantly increased anthocyanins (g^{-1} DM) concentration by 46% in season one at vegetative stage and 61% and 59% in season two at vegetative and flowering stage respectively (Table 2). In the screen house experiment, anthocyanins (g^{-1} DM) concentration increased by 91% as a result of stress in flowering stage (Table 3). Anthocyanins (g^{-1} DM) concentrations were significantly more pronounced in varieties F8 Drought Line, F9 Kidney Selection and JESCA in season one under vegetative stage (Table 2). However variety F8 drought line shows significant increase in anthocyanins concentration as compared with the other studied varieties in season one at flowering stage in field experiment (Table 2).

Significant increase in anthocyanins (g^{-1} DM) content was recorded in varieties F9 Kidney Selection, F8 Drought Line and JESCA as compared with varieties KAT B9 and KAT B1 in season two under field experiment (Table 2).

In the screen house experiment, anthocyanins concentrations was higher in variety F8 Drought Line in vegetative stage and variety KAT B1 in flowering stage (Table 2).

Table 2. Effects of inoculation with *R. leguminosarum,* water stress and five *P. vulgaris* (L.) varieties on the accumulation of Anthocyanins (g^{-1} DM) in common bean shoots for two consecutive season's field experiment.

	1st Season		2nd Season	
Growth Phases	Vegetative	Flowering	Vegetative	Flowering
Treatments inoculation				
R+	0.21±0.01a	0.06±0.007b	0.11±0.009b	0.14±0.01a
R-	0.22±0.02a	0.21±0.02a	0.21±0.04a	0.17±0.02a
Stress Levels				
S_1	0.15±0.007b	0.13±0.01a	0.09±0.01b	0.09±0.01b
S_2/S_3	0.28±0.01a	0.15±0.03a	0.23±0.03a	0.22±0.02a
Varieties				
V_1	0.15±0.02d	0.08±0.02c	0.09±0.01b	0.12±0.01c
V_2	0.19±0.02cd	0.10±0.02bc	0.10±0.01b	0.12±0.01bc
V_3	0.24±0.03ab	0.12±0.02bc	0.23±0.06a	0.19±0.05a
V_4	0.27±0.03a	0.22±0.06a	0.22±0.06a	0.18±0.03ab
V_5	0.22±0.02bc	0.17±0.04ab	0.16±0.03ab	0.16±0.02abc
3-Way Anova (F-Statistics)				
Rhz	1.55ns	48.03***	9.10**	1.81ns
StrL	92.49***	0.88ns	19.47***	39.63***
Vrty	8.79***	5.33**	3.59*	2.35ns
Rhz*StrL	1.18ns	1.79ns	3.19ns	1.71ns
Rhz*Vrty	0.36ns	1.16ns	1.65ns	0.66ns
StrL*Vrty	0.81ns	1.30ns	1.12ns	1.18ns
Rhz*StrL*Vrty	0.15ns	0.78ns	0.68ns	0.57ns

+R: With *R. leguminosarum;* −R: Without *R. leguminosarum*; S_1: No water stress. S_2: Water stress at Vegetative Stage. S_3: Water stress at Flowering Stage. V_1: *KAT B9*. V_2: *KAT B1*. V_3: *F9 Kidney Selection*. V_4: *F8 Drought Line*. V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 3. Effects of inoculation with *R. leguminosarum,* water stress and five *P. vulgaris* (L.) varieties on the accumulation of Flavonoids (g^{-1} DM) and Anthocyanins (g^{-1} DM) in common bean shoots grown in the screen house.

Growth Phases	Vegetative		Flowering	
Treatments *inoculation*	Flavonoids (g^{-1} DM)	Anthocyanins (g^{-1} DM)	Flavonoids (g^{-1} DM)	Anthocyanins (g^{-1} DM)
R+	2.65±0.05b	0.42±0.005b	1.92±0.14a	0.23±0.03b
R-	2.73±0.02a	0.45±0.009a	1.86±0.15a	0.25±0.03a
Stress Levels				
S_1	2.68±0.03a	0.44±0.007a	1.06±0.09b	0.04±0.009b
S_2/S_3	2.71±0.04a	0.44±0.008a	2.72±0.03a	0.44±0.007a
Varieties				
V_1	2.37±0.10c	0.39±0.005e	1.52±0.27c	0.22±0.04b
V_2	2.69±0.02b	0.41±0.03d	1.71±0.26b	0.26±0.05a
V_3	2.73±0.01ab	0.43±0.005c	1.85±0.24b	0.25±0.05ab
V_4	2.82±0.02a	0.50±0.02a	2.19±0.16a	0.24±0.05ab
V_5	2.84±0.02a	0.45±0.004b	2.18±0.17a	0.24±0.04ab
3-Way Anova (F-Statistics)				
Rhz	4.49*	27.06***	0.98ns	4.83*
StrL	0.58ns	0.05ns	780.26***	1276.32***
Vrty	17.50***	46.20***	20.12***	1.31ns

Growth Phases	Vegetative		Flowering	
Treatments *inoculation*	Flavonoids (g^{-1} DM)	Anthocyanins (g^{-1} DM)	Flavonoids (g^{-1} DM)	Anthocyanins (g^{-1} DM)
Rhz*StrL	0.26ns	2.11ns	0.84ns	0.70ns
Rhz*Vrty	4.48**	5.49***	0.21ns	1.03ns
StrL*Vrty	0.66ns	0.72ns	8.45***	1.01ns
Rhz*StrL*Vrty	0.33ns	2.10ns	0.38ns	1.28ns

+R: With *R. leguminosarum*; –R: Without *R. leguminosarum*. S_1: No water stress. S_2: Water stress at Vegetative Stage. S_3: Water stress at Flowering Stage. V_1: KAT B9. V_2: KAT B1. V_3: F9 Kidney Selection. V_4: F8 Drought Line. V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$ at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Interactive effects of inoculation with *R. leguminosarum bv. phaseoli* and stress period on flavonoids (g $^{-1}$ DM) and anthocyanins (g $^{-1}$ DM) in selected *P. vulgaris* (L.)

There were significant interactions between *R. leguminosarum bv. phaseoli*, stress period and varieties in shoot flavonoids and anthocyanins (g^{-1} DM) concentrations in both fields and screen house experiment (Fig. 1-5). Generally, the interactive effects between water stressed, rhizobial inoculated treatments and varieties had a significant effects in flavonoids and anthocyanins concentrations (Fig. 1 - 5).

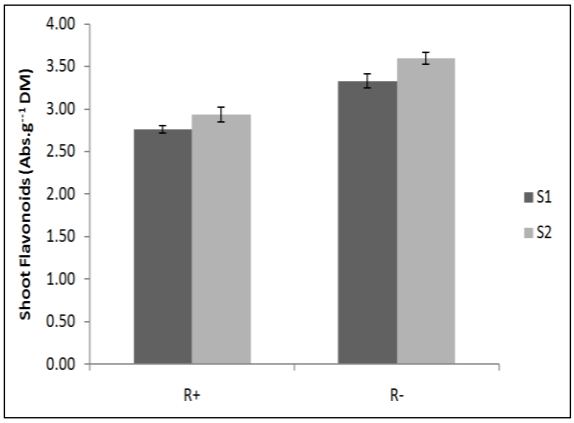

Fig. 1. Interactive effects of *R. leguminosarum bv. phaseoli* and stress level on shoot flavonoids concentration in season (1) field experiment under vegetative stage (+R-: With *R. leguminoserum*, -R-: Without *R. leguminoserum*, S1-: Control, S2-: Water stress at vegetative stage).

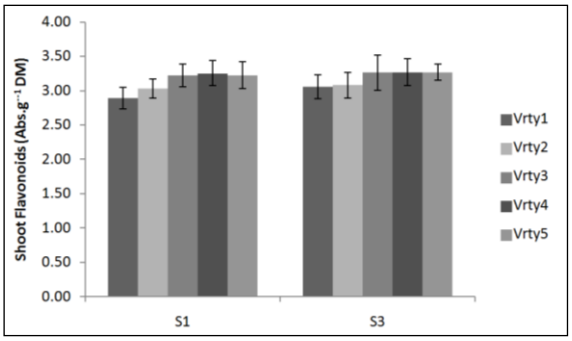

Fig. 2 Interactive effects of stress level and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in season (1) field experiment under flowering stage (S1-: Control, S3-: Water stress at flowering stage**:** Vrty1-: KAT B9, Vrty2-: KAT B1, Vrty3-: F9 Kidney Selection, Vrty4-: F8 Drought Line, Vrty5-: JESCA).

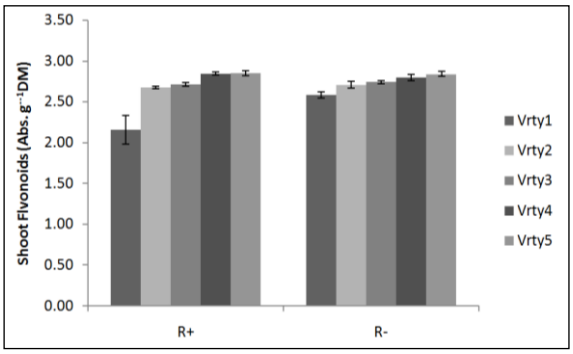

Fig. 3 Interactive effects of *R. leguminosarum bv. phaseoli* and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in screen house experiment under vegetative stage (+R-: With *R. leguminoserum*, -R-: Without *R. leguminoserum*, Vrty1-: KAT B9, Vrty2-: KAT B1, Vrty3-: F9 Kidney Selection, Vrty4-: F8 Drought Line, Vrty5-: JESCA).

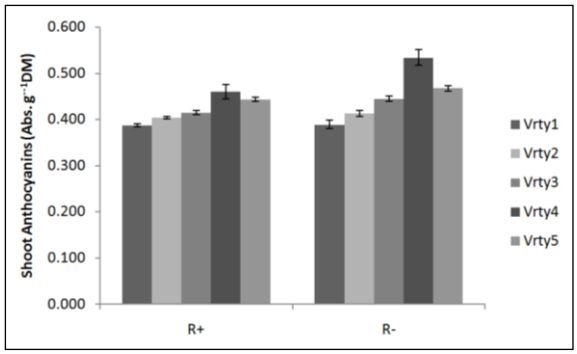

Fig. 4 Interactive effects of *R. leguminosarum bv. phaseoli* and five (5) *P. vulgaris* (L.) on shoot anthocyanins concentration in screen house experiment under vegetative stage (+R-: With *R. leguminosarum*, -R-: Without *R. leguminosarum*, Vrty1-: KAT B9, Vrty2-: KAT B1, Vrty3-: F9 Kidney Selection, Vrty4-: F8 Drought Line, Vrty5-: JESCA).

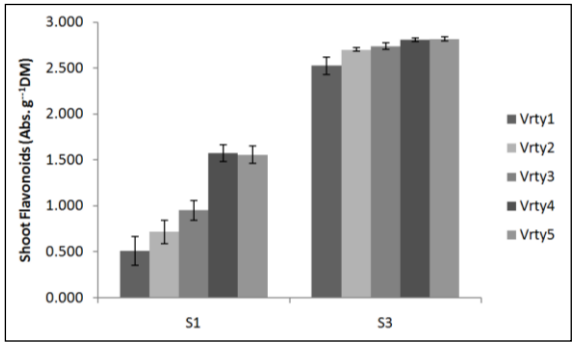

Fig. 5 Interactive effects of stress level and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in screen house experiment under flowering stage (S1-:Control, S3-: Water stress at flowering stage :, Vrty1-: KAT B9, Vrty2-: KAT B1, Vrty3-: F9 Kidney Selection, Vrty4-: F8 Drought Line, Vrty5-: JESCA).

Discussion

Rhizobial inoculation significantly reduced the secondary metabolites (i.e. flavonoids and anthocyanins) in bean shoots at all seasons with field and screen house experiments (Table 1, 2 & 3). The low concentration of these metabolites under rhizobial inoculation suggests that plants were not nutritionally stressed by nitrogen and hence lower accumulation of the secondary metabolites in their tissue. Similar to our study, Makoi *et al.* (2010) showed a decreased level of flavonoids and anthocyanins concentration in *P. vulgaris* (L.) shoots both in fields and screen house experiment inoculated with rhizobial. Scientific evidence has revealed that flavonoids are synthesized using phenylalanine pathway which may be affected by nitrogen metabolism (Laurentius *et al.*, 2002). Under conditions of low N, the levels of Phenylalanine ammonia-lyase (PAL) activity increase hence increasing accumulation of flavonoids (Stewart *et al.*, 2001; Mierziak *et al.*, 2014). Study by Liu *et al.* (2010) in *C. morifolium* leaves showed that flavonoids concentrations were low under higher nitrogen supply. Therefore, the reduced levels of flavonoids and anthocyanins` in this study in the inoculated treatments may be due to enhanced nitrogen fixation and reduced nitrogen stress in the plant.

There was significance increase in flavonoids and antocyanins (g^{-1} DM) concentration in water stress treatment as compared with un-stressed water treatment. Several studies have shown that many of secondary compounds are commonly accumulated in plant tissues in response to various environmental stresses such as water stress and/or drought (Balakumar *et al.*, 1993; Barnabas *et al.*, 2008; Farooq *et al.*, 2009; Odjegb *et al.*, 2013). Synthesis of these compounds stands as a defensive mechanism of plant metabolites such as sugars, proteins, amino acids, nucleic acids, membrane and lipids against reactive oxygen species (ROS), thus serving as an indicator of tolerance to water deficiency in plants (Larson, 1988; Agati *et al.*, 2012). In closely related studies, significant increases in flavonoids and anthocyanins in plant tissues were reported as a result of water stress in various crop plants (Fini *et al.*, 2011; Chalker-Scott, 1999). For instance, drought stress significantly increased anthocyanins levels in cowpea seedlings (Balakumar *et al.*, 1993) a phenomenon similar to our study.

Generally, the results obtained in this study showed variations in the accumulation of flavonoids and anthocyanins. Varieties F8 Drought Line, JESCA and F9 Kidney Selection) significantly contained more flavonoids and anthocyanins as compared with the other studied varieties.

Accumulation of these secondary metabolites in plant tissues has been established as a tolerance mechanism towards several abiotic stresses including water (Larson, 1988; Bergman, 1992; Bongue-Bartelsman and Phillips, 1995; Di Ferdinando *et al.*, 2012; Di Ferdinando *et al.*, 2014; Mazid *et al.*, 2011; Zadehbagheri, 2014).

This confirms the previous finding which reported that bean variety JESCA was able to withstand moderate salinity in a potted study (Ndakidemi and Makoi, 2009), and varieties F8 Drought line, JESCA and KAT B1 accumulated significantly higher amounts of proline in their tissues (Tairo *et al.*, 2017) and hence indicating their potential in drought tolerance studies.

The significantly higher amount of flavonoids and anthocyanins concentration in the mentioned varieties provide a room for further detailed studies related to drought and/or water stress in *P. vulgaris* (L.). Significant interaction was also observed between rhizobial inoculation, water stress and varieties. Highest flavonoids values were recorded in water stressed treatments which were not inoculated with rhizobial inoculants, indicating that stress levels were key in controlling the biosynthesis of flavonoids in the *P. vulgaris* (L.) shoots.

Conclusion

In conclusion, these results showed that flavonoids and athocyanins concentrations (g $^{-1}$ DM) were higher in non rhizobial inoculated treatments as compared with inoculated plots. Furthermore, water stress treatments significantly accumulated more of flavonoids and anthocyanins as compared with unstressed treatments. The accumulation of flavonoids and anthocyanins in plant tissues may be taken as a mechanism used by plants against water deficit. Varieties F8 Drought Line, JESCA and F9 Kidney Selection consisted higher concentrations of flavonoids and anthocyanins as compared with other studied cultivars. These results suggests that flavonoids and anthocyanins are released when plants are subjected to nutritional and water stresses such as those evaluated in this study.

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