Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 105-108
Received: 16-11-2017
Accepted: 21-12-2017

Shuba AC
Department of seed science and technology, UAS. Bengaluru University of Agricultural Sciences, Bengaluru, Karnataka, India

Channnakeshava BC
Department of seed science and technology, UAS. Bengaluru University of Agricultural Sciences, Bengaluru, Karnataka, India

Bhanuprakash K
Indian Institute Horticultural Research, Division of Seed Science and Technology, Karnataka, India

Arvind Kumar
Department of seed science and technology, UAS. Bengaluru University of Agricultural Sciences, Bengaluru, Karnataka, India

Correspondence
Shuba AC
Department of seed science and technology, UAS. Bengaluru University of Agricultural Sciences, Bengaluru, Karnataka, India

Study on seed quality performance and enzymatic activity after dormancy breaking in henna

Shuba AC, Channnakeshava BC, Bhanuprakash K and Arvind Kumar

Abstract
Henna (*Lawsonia inermis* L.) is a important medicinal plant belonging to the family Lythraceae. Seeds of this species exhibit poor germination and posses seed dormancy. In an effort to find out suitable method to break dormancy to improve germination and promote the cultivation of this plant, treatments were imposed Viz., GA_3 @ 500 ppm (24 hr and 48hr), KNO_3 @ 1% (24hr and 48hr), wet stratification @ 10° C (24hr and 48hr), soaking in water (24hr and 48hr) acid scarification (3min and 6min), mechanical scarification by rubbing with sand paper (3 min and 6 min) and dry seeds were kept as control (not treated). The highest germination (92.25 %), mean seedling length (5.40 cm), mean seedling dry weight (6.83 mg), SVI-I (498) and SVI –II (630) was observed in the treatment KNO_3 24 hr. Whereas lowest germination (31.23%), mean seedling length (2.00 cm), mean seedling dry weight (4.01 mg), SVI-I (63) and SVI–II (125) was observed in the treatment acid scarification 6 min. The highest α- Amylase activity (64.97 µg g^{-1}) was recorded in the treatment KNO_3 24hr and lowest activity (43.72 µg g^{-1}) was recorded in dry seeds (control – dormant seeds). The dormancy breaking treatments from 1 to 5 were scored as low, medium, high intensity and very high intensity with Rm value ranged from 0.0178 to 0.0948 for esterase activity.

Keywords: henna, dormancy breaking, seed germination, α- amylase activity, esterase activity

Introduction
Medicinal plants are valuable natural resources. They have been identified and used throughout human history. Henna have high medicinal value and are an important source for pharmaceutical industries. To conserve germplasm, to meet demand and to avoid overexploitation as the cultivation is limited, its needed to protect from line of extinction. Thus commercial cultivation is the need of hour which is hindered by problems of poor germination due to seed dormancy when attempted for cultivation. Also there is limited information concerning germination improvement or dormancy breaking methods and on work of enzyme activity associated with dormancy treatments. Thus objective of this study was to develop some effective method that would help to break dormancy and provide good plant stand and yield.
Henna (*Lawsonia inermis* L.) is a member of the family Lythraceae. It is also commonly called as "Mehandi". Its origin is unknown but linguistic evidence support the origin in tropic and subtropics of Baluchisthan. The Commercial production of henna is limited to few places in India, Pakistan, Iran, Egypt and Sudan. Henna bark, leaves and seeds are used for medicinal purpose (Amit *et al*., 2011) [2].
Henna consists of various categories of phytoconstituents like flavonoids, coumarine, triterpenoids, steroids and xanthones. In Shushrutha Samhita it has been recommended as a remedy for maligan ulcers. Leaves are used as diuretic, for headache, prickly heat (as a cooling agent) and for skin disease. In Charaka Samhita, it has been described as useful for treating epilepsy and jaundice (Amit *et al*., 2011) [2]. Its leaves and aerial parts are used for treating wounds, ulcers, cough, bronchitis and jaundice (Shiharta *et al*., 1978) [12].
Henna is a much branched, deciduous, glabrous, sometimes a shrub or a small tree, with greying brown bark, attaining a height of 2.4m-5m. Leaves are elliptic, acute and petioles are very short. Flowers are numerous less than 1.3cm and they are white or rose coloured in large terminal pyramidal panical or cyme, pedicels short, calyx companulate. Capsule 6 mm diameter, slightly veined outside suported by persistant calyx and tipped with style. Seed capsules are red, glabose, with numerous tiny pyramidal brown pitted seed (Amit *et al*., 2011) [2].

Material and methods
Source of seeds
Freshly harvested seeds of henna were collected from the "Sanjeevini Vatika" aromatic and medicinal plants garden in the Department of Horticulture, UAS, GKVK, Bengaluru.

The seeds obtained from the source were cleaned and dried to a safer moisture content and graded to uniform size and were later used for the study.

Experimental details

Substrata: Top of paper (TP),

Temperature: 20/30 ºC, Replications – four

Seeds were treated with GA_3 @ 500 ppm (24 hr and 48hr), KNO_3 @ 1% (24hr and 48hr), wet stratification @ 10 ^{0}C (24hr and 48hr), soaking in water (24hr and 48hr) acid scarification (3min and 6min), mechanical scarification by rubbing with sand paper (3 min and 6 min) and dry seeds were kept as control (not treated).

Observations recorded for dormancy breaking treatments

The germination test was conducted in the laboratory by using top of paper method as per ISTA rules (Anon., 1985), Speed of germination was calculated from Bartlett's Rate Index (Bartlett, 1973), Mean germination time (MGT) was calculated according to Ellis and Robert (1981) [4] and expressed in days, seedling vigour index I and II as suggested by Abdul Baki and Anderson (1973) [1], mean seedling length, mean seedling dry weight were also recorded.

Based on the above results the best treatments were selected and estimation of amylase activity as per Sadashivam and Manickam (1996) [11] and esterase was analysed as described by Gennady (1994) [5] and Glaszman *et al*. (1988) [6] by keeping dry seeds as control.

Statistical analysis

The statistical analysis and the interpretation of the experimental data was done by using Fisher's method of analysis of variance technique as outlined by Gomez and Gomez (1984) [7] and the level of significance at one percent for CRD design.

Result and discussion

The significant difference was observed among the different dormancy breaking treatments imposed in henna crop. The highest germination (92.25%), mean seedling length (5.40 cm), mean seedling dry weight (6.83 mg), SVI-I (498) and SVI –II (630) in the treatment KNO_3 24 hr. Whereas lowest germination (31.23%), mean seedling length (2.00 cm), mean seedling dry weight (4.01 mg), SVI-I (63) and SVI –II (125) in the treatment acid scarification 6 min. (Table 1).

Speed of germination (0.6552) was recorded highest in water 48 hr and lowest (0.5505) was recorded in treatment acid scarification 6 min. While Mean germination time was recorded lowest (8.85 days) in water 48 hr and highest (10.53 days) was recorded in acid scarification 6 min. (Table 1).

Light is an extremely important factor for releasing seeds from dormancy. Seeds of many species are affected by exposure to white light for a few minutes or seconds. Whereas, others require intermittent illumination, light requiring seed under long-term illumination with white light could break the dormancy. KNO3 is most widely used chemical for promoting germination of seeds which are sensitive to light. The stimulatory effect of KNO3 influence the respiratory system directly and also by stimulating oxygen uptake (Hilton and Thomas, 1986) [9] or serve as a co-factor of phytochrome, a light sensitive protein pigment (Hilhorst, 1990) [8].

Table 1: Effect of seed dormancy breaking treatments on seed quality of henna (*Lawsonia inermis* L.)

Treatments	Germination (%)	Mean seedling length (cm)	Mean seedlings dry weight (mg)	Seedling vigour index - I	Seedling vigour index -II	Speed of germination	Mean Germination time (days)
T_0: Control	73.50	4.30	5.66	316	416	0.5699	10.32
T_1: GA_3 24 hr	79.24	4.50	5.81	357	460	0.6305	10.13
T_2: GA_3 48 hr	77.20	4.10	5.37	317	415	0.5880	9.44
T_3: KNO_3 24 hr	92.25	5.40	6.83	498	630	0.6521	9.16
T_4: KNO_3 48 hr	90.26	5.10	6.52	460	588	0.5916	9.51
T_5: Water 24 hr	87.24	3.40	4.97	297	434	0.5811	9.34
T_6: Water 48 hr	84.27	3.70	4.61	312	388	0.6452	8.85
T_7: Wet stratification 24 hr	75.26	4.90	5.68	369	427	0.5791	9.31
T_8: Wet stratification 48 hr	69.21	4.30	5.21	298	361	0.6059	9.74
T_9: Acid scarification (3 min)	37.19	2.90	4.08	108	152	0.5523	8.88
T_{10}: Acid scarification (6 min)	31.23	2.00	4.01	63	125	0.5505	10.53
T_{11}: Mechanical scarification (3 min)	46.18	3.10	4.32	143	200	0.5652	9.09
T_{12}: Mechanical scarification (6 min)	43.14	2.35	4.13	101	179	0.6046	9.72
S. Em±	0.49	0.01	0.02	2	3	0.001	0.01
CD (p=0.01)	1.41	0.03	0.05	6	8	0.003	0.04
CV	1.46	0.77	0.24	2	1	0.344	0.29

α- Amylase activity (µg g^{-1})

In henna, there was significant difference between treated and untreated seeds (dry). The highest α- Amylase activity (64.97 µg g-1) was recorded in the treatment KNO3 24hr and lowest activity (43.72 µg g-1) was recorded in dry seeds which was a control. (Table 2).

Table 2: Effect of dormancy breaking treatments on α –amylase activity (µg g-1) in henna

Treatments	Primrose
T_1: GA_3 (24 hr)	50.29
T_7: Wet stratification 10 ºC (24 hr)	53.95
T_3: KNO_3 (24 hr)	64.97
T_5: Soaking in water (24 hr)	60.24
T_o: Control	43.72
S.Em±	0.714
CD	2.18

Banding intensity and relative mobility of esterase (EST) isozyme of henna seeds

The presence or absence of specific band or group of bands as well as band intensity was taken as the criteria to characterize the esterase profile extracted from seeds subjected to dormancy breaking treatments in henna. The dormancy breaking treatments from 1 to 5 were scored as low, medium, high intensity and very high intensity with Rm value ranged from 0.0178 to 0.0948. All the dormancy breaking treatments showed significant differences either by the presence or absence of bands and as well as their intensity. (plate 1, plate 2 and table 3).

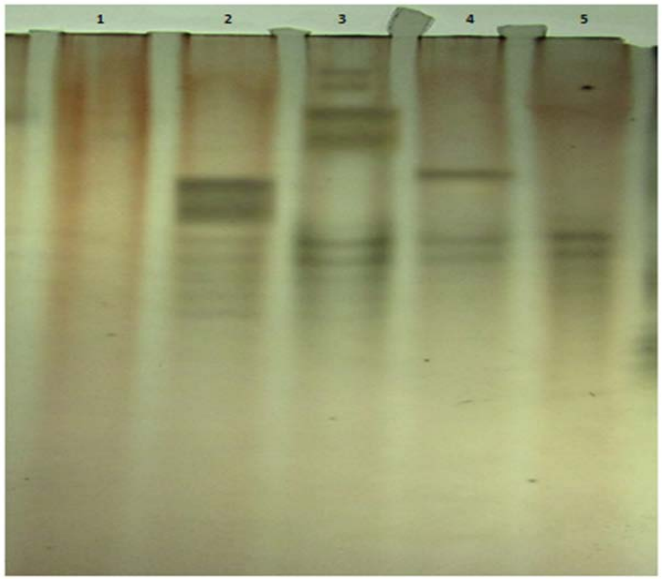

Plate 1: Banding pattern of esterase profile

Treatment details

1. Dry seeds –control – T_0
2. Water soaking – 24hr – T_5
3. KNO_3 – 24hr- T_3
4. GA $_3$ 24hr – T_1
5. Wet stratification 24hr – T_7

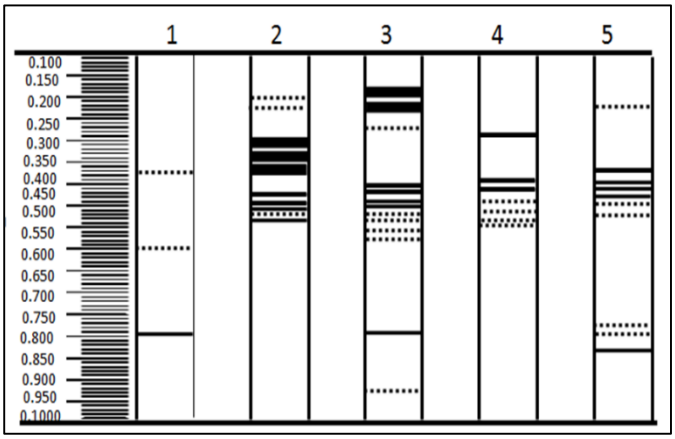

Plate 2: Zymogram of esterase activity in henna

Treatment details

1. Dry seeds –control – T_0
2. Water soaking – 24hr – T_5
3. KNO_3 – 24hr- T_3
4. GA $_3$ 24hr – T_1
5. Wet stratification 24hr – T_7

Table 3: Banding intensity and relative mobility of esterase (EST) isozyme of henna seeds

Band No.	Rm value	1	2	3	4	5
1	0.0147			++++		
2	0.0156	++++	++++	++++		
3	0.0249	+				
4	0.0268			+		
5	0.0295		+++	+++	+++	
6	0.0332	+++				
7	0.0349	+++				
8	0.0691	+++		+++	++	+++
9	0.0702	+++	+++	+++	+++	++
10	0.0735	++	+++	+++	+	++
11	0.0749	+++		+++	+	+
12	0.0768			++		+
13	0.0812		+			
14	0.0839		++		++	
15	0.0862				+	
16	0.0867				++	

			++	:	Low
++++	:	High	+	:	Very Low
+++	:	Medium	Absent	:	No band

Treatment details

1. Dry seeds –control – T_0
2. Water soaking – 24hr – T_5
3. KNO_3 – 24hr- T_3
4. GA $_3$ 24hr – T_1
5. Wet stratification 24hr – T_7

Band No. 1: It was differentiated into two types of bands based on intensity with Rm value of 0.0000 to 0.0178. Very high intensity band was spotted in T4 and low intensity band was noticed in T8. However it was absent in remaining treatments.

Band No. 2: It was characterized in to two types of bands based on intensity with Rm value of 0.0178 to 0.0232. Very high intensity band was spotted in T4 and low intensity bands were observed in T8 and T6. However it was absent in remaining treatments.

Band No. 3: It was composed of two types of bands based on intensity with Rm value of 0.0232 to 0.0265. High intensity band was spotted in T1 and low intensity band was observed in T4. However it was absent in remaining treatments.

Band No. 4: It was composed of single type of band *i.e.*, very high intensity band with an Rm value of 0.0265 to 0.0304 present only in T8. It was absent in remaining treatments.

Band No. 5: It was characterized into three types of bands based on intensity with Rm value of 0.0304 to 0.0374. Very high intensity band was spotted in T8 while high intensity bands were observed in T4, T1 and T6. Low intensity band was observed in T0.

Band No. 6: It was composed of two types of bands based on intensity with Rm value of 0.0374 to 0.0453. High intensity band was spotted in T8, T4 and T1. Medium intensity band was observed in T6. However it was absent in T0.

Band No. 7: It was characterized into three types of bands based on intensity with Rm value of 0.0453 to 0.0478. High

intensity band was noticed in T8 while medium intensity bands were observed in T4 and T6. Low intensity band was observed in T1.

Band No. 8: It was composed of single type of band *i.e.*, low intensity band with an Rm value of 0.0478 to 0.0512 present in T8, T4, T1 and T6. It was absent in T0.

Band No. 9: It was composed of two types of bands based on intensity with Rm value of 0.0512 to 0.0523. Medium intensity band was observed in T8 and low intensity bands were observed in T4, T1 and T6. However it was absent in T0.

Band No. 10: It was composed of single type of band *i.e.*, low intensity band with an Rm value of 0.0523 to 0.0590 present in T4 and T1. However, it was absent in T0, T8 and T6.

Band No. 11: It was composed of single type of band *i.e.*, low intensity band with an Rm value of 0.0590 to 0.0613 present only in T0 and absent in T8, T4, T1 and T6.

Band No. 12: It was consists of single type of band *i.e.*, low intensity band with an Rm value of 0 0.0613 to 0.0704 present only in T6 and absent in T8, T4, T1 and T0.

Band No. 13: It was composed of two types of bands based on intensity with Rm value of 0.0704 to 0.0812. Medium intensity bands were observed in C2T0 and C2T4 whereas low intensity band was observed in T6. However it was absent in T8 and T1.

Band No. 14: It was consists of single type of band *i.e.*, low intensity band with an Rm value of 0 0.0812 to 0.0948 present only in T4 and absent in T8, T6, T1 and T0.

The banding profile of esterase isozyme showed polymorphism among dormancy breaking treatments with the presence or absence of bands. There was increase in the intensity of the bands after subjecting to dormancy breaking treatments. Thus we can conclude that there was positive correlation between dormancy breaking treatments and enzyme machinery activation. Since esterases are necessary for the breakdown of storage lipids in to fatty acid, which provide the biosynthetic energy necessary for embryo to push the essential structures, alteration in isoenzyme profiles noticed in the crop indicate that the pattern of enzyme polymorphism changes during dormancy breaking.

Summary

The present investigation revealed that treatment KNO_3 24 hr followed by KNO_3 48 hr was effective in breaking dormancy and improving the germination percentage and other quality parameters. With respect to esterase profile indicated that after dormancy breaking there was increase in machinsery activation and breakdown of storage lipids. Alpha amylase activity was also noticed highest in KNO_3 24 hr which indicated the breakdown and utilization of substrates for germination and growth of seedlings, hence treating henna seeds in KNO_3 24 hr and then sowing find its practical utility.

References

1. Abdul Baki AA, Anderson DJ. Vigour determination in soybean seed bymultiple criteria. Crop Sci. 1982; 13:630-633.
2. Amit S, Borade, Babasaheb N, Kale Rajkumar shete V. A Phytopharmacological review on *Lawsonia inermis* (Linn.). Int. J Pharmacy and Life Sci. 2011; 2(1):233.
3. Bartlett MS. Some examples of statistical methods of research in applied biology (Suppl.). J Res. Stat. Soc. 1973; 4(2):137-183.
4. Ellis RA, Roberts EH. The quantification of ageing and survival of orthodox seeds. Seed Sci. and Technol. 1981; 9:373-409.
5. Gennady P, Manichenko. Handbook on detection of enzymes on electrophoresis gel. II edition CRC press, Inc. Boca Raton, USA, 1994.
6. Glaszman JC, Delos Reyes BG, Khush GS. Electrophoretic variation of isozymes in plumules of rice. IRRI Res. Paper series. 1988; 134:1-3.
7. Gomez KA, Gomez AA. Statistical procedure for Agric. Res., 2nd Ed. John Wiley and Sons, New York, 1984.
8. Hilhorst HWM. Dose response analysis of factors involved in germination and secondary dormancy in seeds of *Sisymbrium officinale*. Plant Physiology. 1990; 94:1096-1102.
9. Hilton JK, Thomas JA. Regulation of pre-germination rates of respiration in seeds of various seed species by potassium nitrate. J Exptl. Bot. 1986; 37:1516-1524.
10. ISTA. International rules for seed testing. Seed Sci. and Technol. 1985; 13:299-513.
11. Sadasivam S, Manickam A. Biochemical methods. New Age International, New Delhi. 1996, 1-21.
12. Shiharta IM, Hussain AG, Mayah GY. Pharmacological effects of *Lawsoniainermis* leaves (elhenna). Egypt J Vet. Sci. 1978; 15:31.