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Studies on seed quality attributes in shankapushpi (*Clitorea ternatea* L.)

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Abstract
The experiment was conducted to study the effect of media, temperature, dormancy breaking treatments and biochemical changes associated with dormancy on seed quality of shankapushpi. The experiment results revealed that between paper method at 30^0C recorded highest germination (59.75%), seedling vigour Index-I (1277) and seedling vigour index-II (9700) in shankapushpi seeds. Among different dormancy breaking treatments, mechanical scarification (2side) recorded the highest seed germination (88.50%), seedling vigour Index-I (2423) and seedling vigour index-II (16583) respectively. With respect to different biochemical changes associated, highest α- Amylase activity (69.21 µg g^{-1}) was recorded in the treatment mechanical scarification (2 sides). The banding pattern of esterase isozyme profile showed significant difference for different dormancy breaking treatments either by presence or absence of bands as well as intensity their with Rm values ranged from 0.0158 to 0.0871.

Keywords: Attributes, shankapushpi, *Clitorea ternatea* L. medicinal plants

Introduction
Medicinal plants also form a group of important plants and are valuable as they produce different kinds of alkaloids, glycosides, sterols and essential oil etc. as secondary products of plant Metoblism. These secondary metabolites are used in medicine to cure various ailments as herbal remedies for health care and are basic raw material for synthetic drugs and antibiotics in pharmaceuticals. But potential utility and commercial cultivation of medicinal plants has not taken place due to lack of knowledge among farmers, lack of research work in enhancing seed viability, germination and overcoming dormancy.

So, there is a need to study the problems in their seed germination and seed dormancy in order to uplift the seed quality for better and quick germination. To address the above acute problems, this study was undertaken to standardize seed germination procedure and to evaluate the effective method for breaking dormancy to provide good plant stand and yield as well as to study biochemical/enzymatic activity changes during dormancy breakdown.

Sankapushpi (*Clitorea ternatea*) belonging to the family Fabaceae has a wide range of medicinal application in ayurvedic pharmacopoeia. It is also commonly called as Aparanjitha, Butterfly pea and Asian pigeon wings. It is native to Tropical equatorial Asia but is grown world wide. It is commonly called as Shankapushpi in sanskrit where it is reported to be a good 'Medhya'(brain tonic) as memory enhancer and for treating mental illness (Manju Lata Zingare *et al*., 2013) [10]. The major phyto-constituents found in Shankapushpi are the pentacyclic triterpenoids such as taraxerol, tarazerone, ternatins and tannins. It is reported to have tranquilising property, anti inflammatory, antipyretic, antioxidant, antidiabetic, heat protective and immunomodulatory activities (Mukharjee *et al*., 2008) [11].

The flavanol and glycoside present in its roots is reported to have antibacterial activity. Roots, seeds and flowers have medicinal values and are used to treat various ailments like headache, infection and urogenital disorder. Juice of flowers is reported to be used in insect bites and skin diseases and the roots are useful in treating inflammation, leucoderma, leprosy, pulmonary tuberculosis etc. Seeds are cathartic and are useful in viserdlgia (Manju Lata Zingare *et al*., 2013) [10].

Shankapushpi has twining fine stem, 0.5- 3 m long, leaves are pinnate, 3-5 cm long, elliptic to laminate leaflets and shortly pubescent underneath. Flowers are solitary, deep blue or white, very short pedicellate and 4-5 cm long, pods are flat, linear, beaked, 6-12 cm long and slightly pubescent with upto 10 seeds. Seeds are olive brown to black in colour, often motted with 4.5-7 mm long (Manju Lata Zingare *et al*., 2013) [10].

Materials and Methods

The experiment was conducted during the year 2015 -16 in Department of Seed Science and Technology, College of Agricultural Sciences, Bengaluru, India. Freshly harvested seeds of Shankapushpi were collected from the Foundation for Revitalisation of Local Health Traditions (FRLHT), Yelahanka. 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. For standardization of seed germination procedure, the experiment was laid out in FCRD with four replications consisting of two factors i.e., main factor substrata(S) with sub factors such as S1: Top of paper (TP), S2: Between paper (BP), S3: Sand media (S), S4: Quartz sand (QS), S5: Pleated paper (PP) and another main factor temperature(T) with sub factors such as T1: 20±1°C, T2: 30±1°C and T3: 20/30±1°C respectively.

From the above experiment best substrata and temperature was choosen to conduct experiment of imposing different dormancy breaking treatments such as GA3 @ 500 ppm (24 hr and 48hr), KNO3 @ 1 % (24hr and 48hr),wet stratification @ 100 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) with four replications laid out in CRD design to evaluate seed quality parameters. The following observations were recorded *viz*., the germination test was conducted in the laboratory by using between paper method as per ISTA rules (Anon., 1985), 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) [13] 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

1. To standardize the seed germination procedure for Shankapushpi (*Clitorea ternatea* L.)

1.1 Influence of substrata on seed germination and seedling characters

In shankapushpi significantly highest germination percentage, first count, final count and abnormal seedlings (54.33 %, 37.90 %, 65.08 % and 8.00) was recorded in between paper (S2). Whereas, lowest (32.17 %, 29.58 % and 46.25 % and 6.50, respectively) noticed in top of paper method (S1). Lower number of hard and diseased seeds were found (10.42 and 9.17) in pleated paper (S5). Whereas, the highest number (14.08 and 13.42 respectively) in top of paper method (S1).Minimum number of FUGs and dead seeds number found (10.00 and 6.92) in between paper method (S2).Whereas, the highest number (20.58 and 10.92, respectively) in top of paper method (S1) (Table 1). The mean seedling length, seedling dry weight, SV-I and SVI-II (19.99 cm, 151.90 mg, 1092 and 8295) recorded highest in between paper method (S2). Whereas, the lowest of (14.02 cm, 108.12 mg, 452 and 3482, respectively) in top of paper (S1). (Table 2).

Shankapushpi seeds are bigger in size and also exhibited hard seed coat nature, which performed well in between paper rather than top of paper because of the proper availability of moisture, space for seedling emergence, root and shoot growth which put up more growth inturn results in other higher quality parameters like mean seedling length, mean seedling dry weight etc. These conditions might have caused higher growth of seedlings such results were also reported venudevan *et al.* (2013) [17] in *Aegle marmelos* seeds and Sriram (2004) [16] in *Withania somnifera* seeds.

Table 1: Effect of media and temperature on seed quality of shankapushpi (*Clitoria ternatea* L.)

Treatments	Germination (%)	Hard seeds (Nos.)	Fresh ungerminated seeds (Nos.)	Diseased seeds (Nos.)	Dead seeds (Nos.)	Abnormal seedling (Nos.)	First count (%)	Final count (%)
Medias								
S_1: TP	32.17	14.08	20.58	13.42	12.92	6.50	29.58	66.83
S_2:BP	54.33	10.75	10.00	9.42	6.92	8.00	32.58	75.08
S_3:S	43.75	11.83	14.42	11.33	10.58	7.75	37.92	70.00
S_4:QS	39.50	11.50	17.75	11.42	11.17	7.50	29.92	68.75
S_5:PP	47.75	10.42	15.75	9.17	10.08	6.67	33.25	73.92
S. Em±	0.41	0.18	0.23	0.24	0.24	0.18	0.28	0.52
CD (p=0.01)	1.56	0.67	0.88	0.91	0.90	0.68	1.05	1.96
Temperatures								
T_1: 20 °C	38.20	13.15	16.75	13.00	11.35	7.05	26.65	68.10
T_2:30 °C	48.60	10.60	13.90	9.60	9.15	7.65	37.25	73.10
T_3:20/30 °C	43.70	11.40	16.45	10.25	10.50	7.15	34.05	71.55
S. Em±	0.32	0.14	0.18	0.18	0.18	0.14	0.21	0.40
CD (p=0.01)	1.21	0.52	0.68	0.70	0.70	0.53	0.81	1.52
Interaction (T×S)								
T_1S_1	29.50	16.25	19.75	15.00	13.75	5.50	24.25	65.50
T_1S_2	47.00	13.00	10.75	12.50	7.50	8.25	28.25	70.75
T_1S_3	38.00	13.50	16.25	14.00	9.75	8.25	27.25	67.75
T_1S_4	35.25	12.50	19.00	12.25	13.50	7.00	24.25	66.75
T_1S_5	41.25	10.50	18.00	11.25	12.25	6.25	29.25	69.75
T_2S_1	34.50	12.50	20.25	12.50	13.00	7.25	23.25	67.25
T_2S_2	59.75	10.75	7.75	7.25	6.00	8.50	45.25	78.25
T_2S_3	50.50	9.75	12.50	9.50	10.25	7.50	45.25	72.75
T_2S_4	44.50	9.50	15.00	10.50	9.50	8.50	38.25	69.00

T_2S_5	53.75	10.50	14.00	8.25	7.00	6.50	34.25	78.25
T_3S_1	32.50	13.50	21.75	12.75	12.00	6.75	41.25	67.75
T_3S_2	56.25	8.50	11.50	8.50	7.25	7.25	24.25	76.25
T_3S_3	42.75	12.25	14.50	10.50	11.75	7.50	41.25	69.50
T_3S_4	38.75	12.50	19.25	11.50	10.50	7.00	27.25	70.50
T_3S_5	48.25	10.25	15.25	8.00	11.00	7.25	36.25	73.75
S.Em±	0.71	0.31	0.40	0.41	0.41	0.31	0.48	0.89
CD (p=0.01)	2.70	1.16	1.53	1.57	1.58	1.18	1.82	3.39
CV	3.26	5.20	5.11	7.54	7.96	6.50	2.93	2.52

Medias (S) S1: Top of paper (TP) S2: Between paper (BP) S3: Sand S4: Quartz sand S5: Pleated paper
Temperatures (T) T1: 20 ºC T2: 30 º T3: 20 º/30 ºC

Table 2: Effect of media and temperature on seed quality of shankapushpi (*Clitoria ternatea* L.)

Treatments	Mean seedling length (cm)	Seedling dry weight (mg)	Seedling vigour index - I	Seedling vigour index -II
Medias				
S_1: TP	14.02	108.12	452	3482
S_2: BP	19.99	151.90	1092	8295
S_3: S	19.12	145.31	843	6412
S_4: QS	18.35	139.43	729	5539
S_5: PP	17.33	131.71	831	6321
S.Em±	0.02	0.27	7.15	53.24
CD (p=0.01)	0.06	1.02	27.20	202.51
Temperatures				
T_1: 20 °C	16.60	127.08	642	4912
T_2:30 °C	19.11	145.19	944	7179
T_3:20/30 °C	17.58	133.60	781	5939
S. Em±	0.01	0.21	5.54	41.24
CD (p=0.01)	0.05	0.79	24.07	136.86
Interaction				
T_1S_1	13.43	106.49	396	3140
T_1S_2	18.65	141.75	877	6661
T_1S_3	17.46	132.74	664	5044
T_1S_4	17.07	129.79	602	4575
T_1S_5	16.39	124.64	676	5142
T_2S_1	14.92	113.51	515	3916
T_2S_2	21.37	162.35	1277	9700
T_2S_3	20.91	158.86	1056	8023
T_2S_4	19.81	150.53	882	6698
T_2S_5	18.51	140.69	995	7561
T_3S_1	13.71	104.35	446	3392
T_3S_2	19.95	151.59	1122	8527
T_3S_3	18.99	144.33	812	6170
T_3S_4	18.15	137.97	703	5345
T_3S_5	17.07	129.79	824	6262
S.Em±	0.03	0.46	12	92
CD (p=0.01)	0.10	1.76	41	351
CV	0.30	0.684	3	3

Medias (S) S1: Top of paper (TP) S2: Between paper (BP) S3: Sand S4: Quartz sand S5: Pleated paper
Temperatures (T) T1: 20 ºC T2: 30 ºC T3: 20 º/30 ºC

1.2 Influence of temperature on seed germination and seedling characters

The results on seed germination percentage showed significant difference in shankapushpi. The seed germination, germination at first count and final count were recorded highest (48.60 %, 37.25 % and 59.20 %) at temperature of 30 ºC (T2). Whereas, the lowest (38.20 %, 26.65 % and 51.35 %) was noticed at 20 ºC (T1). The hard, FUGs, diseased and dead seeds were found lowest (10.60, 13.90, 9.60 and 9.15) in temperature of 30 ºC (T2). Whereas, the highest (13.15, 16.75, 13.00 and 11.35, respectively) was noticed at 20 ºC (T1). (Table 1). The mean seedling length, mean seedling dry weight, SVI-I and SVI-II were found highest (19.19.cm, 145.19 mg, 945 and 7180) in temperature of 30 ºC (T2).
Whereas, the highest (16.60 cm, 127.08 mg, 643 and 4913, respectively) was noticed at 20 ºC (T1). (Table 2).

The higher total germination percentage and rate of seed germination and seedling parameters may be due to better water absorption that led to higher biochemical changes resulted in higher rate of germination parameters of seeds at 30 ºC compared to 20/30 ºC. This was in accordance with Ganesh *et al.* (2011) [4] in *Aegle marmelos* seeds which recorded maximum germination at 30 ºC. Bonner (1975) [3] observed same result in *Fraxinus Americana* and Lin *et al.* (1979) [9] in *Phellodendron wilsonii* and *Phellodendron amurense.*

Interaction effect of media and temperature on seed germination and seedling characters

Interaction effects of media and temperature were shown significant difference in shankapushpi. Among the interactions the highest seed germination and final count

(59.75 % and 70.50 %) noticed in between paper at 30 ºC. Whereas, the lowest was recorded (29.50 % and 45.75 %) in top of paper at 20 ºC. Similarly highest germination at first count (45.25 %) was recorded in between paper at 30 ºC. Whereas, the lowest of (23.25 %) recorded in top of paper at 30 ºC. Diseased and dead seeds were found lowest (7.25 and 6.00) in between paper at 30 ºC. Whereas, highest (15.00 and 13.75) on top of paper at 20 ºC. Hard seeds were found lowest (8.50) in between paper at 20/30 ºC. Whereas highest (16.25) on top of paper at 20 ºC. Similarly fresh un-germinated were lowest (7.75) in between paper at 30 ºC and highest (21.75) in top of paper at 20/30 ºC. Abnormal seedlings were lowest (5.50) in top of paper at 20 ºC and highest (8.50) in quartz sand at 30 ºC. (Table 1).

The mean seedling length, SVI-I and SVI-II found highest (21.37 cm, 1277 and 9700) in between paper at 30 ºC. Whereas, the lowest (13.45 cm, 396 and 3140) recorded top of paper at 20 ºC. Similarly, the mean seedling dry weight found highest (162.35 mg) in between paper at 30 ºC. Whereas, the lowest of (104.35 mg) recorded in top of paper at 20/30 ºC. (Table 2).

This may because of the between paper method provides a better oxygen water relationship for initiating the early germination and constant temperature of 30 ºC may initiate the enzymatic activity of the seed. Similar findings were reported by Poojar (2000) [12], who have showed higher values of seedling parameters in between paper method in *Psorelia corylifolia* at 30 ºC.

2. Studies on the effect of seed dormancy breaking treatments in Shankapushpi (*Clitorea ternatea* L.)

There was significant difference observed among the different dormancy breaking treatments imposed in shankapushpi crop. The highest germination (88.50%), mean seedling length (26.88 cm), mean seedling dry weight (187.38 mg), SVI-I (2423) and SVI –II (16583) in the treatment mechanical scarification done on 2 sides. Whereas lowest germination (52.00 %), mean seedling length (17.9 cm), mean seedling dry weight (103.68 mg), SVI-I (930) and SVI –II (5391) in the treatment wet stratification 24 hr. (Table 3).

Scarifying the seed coat simply breaks the dormancy of the seed by exposing the embryo to water. Water uptake is one of the critical factors that active the initial biochemical changes responsible for protein synthesis during germination. Seeds of Fabaceae family exhibit dormancy because of hard testa impermeable to water and gases (Shaik *et al.,* 2008; Ali *et al.,* 2011) [14, 2]. Seed dormancy due to hard seeds is removed by puncturing the seed coat, and the germination increased to 84 per cent. The damage in the leathery testa has allowed the seeds for easy imbibition of water and exchange of gases and softening of seed coat. Further, softened seed coat has allowed the growing tips to emerge out from cotyledons by pushing seed coat apart. Imbibition process initiates the physiological process of germination and results in growth of plumule and radical.

α- Amylase activity (µg g-1)

The results obtained on α- Amylase activity showed significant difference was noticed between treated and untreated seeds (dry), as the highest α- Amylase activity (69.21 µg g-1) was recorded in the treatment mechanical scarification (2 sides) and lowest activity (44.28 µg g-1) was recorded in dry seeds (control – dormant seeds). (Table 4).

The seeds that germinated under the effect of GA3 also showed de novo synthesis of polypeptides that could correspond to some isozymes of alpha-amylase by comparison with alpha-amylase standard. It is very probable that alpha-amylase isozymes play a key role in the mobilization of carbohydrates for a normal growth. It was also reported by Shirin Haddad Kaveh *et al*. (2010) [15] in *F. gummosa* seedlings.

Table 3: Effect of seed dormancy breaking treatments on seed quality of shankapushpi (*Clitorea ternatea* L.)

Treatments	Germination (%)	Mean seedling length (cm)	Mean seedlings dry weight (mg)	Seedling vigour index - I	Seedling vigour index -II
T_0: Control	59.75	21.34	120.08	1275	7175
T_1: GA_3 24 hr	64.75	23.88	149.68	1546	9692
T_2: GA_3 48 hr	60.25	24.08	161.58	1451	9735
T_3: KNO_3 24 hr	54.00	20.48	120.08	1106	6484
T_4: KNO_3 48 hr	59.00	19.68	119.98	1161	7079
T_5: Water 24 hr	61.00	22.68	130.68	1383	7971
T_6: Water 48 hr	68.00	21.78	129.58	1481	8811
T_7: Wet stratification 24 hr	52.00	17.88	103.68	930	5391
T_8: Wet stratification 48 hr	55.75	18.08	115.18	1008	6421
T_9: Acid scarification (3 min)	72.75	24.88	150.08	1810	10918
T_{10}: Acid scarification (6min)	79.00	25.78	151.78	2036	11990
T_{11}: Mechanical scarification (1 side)	84.25	26.88	163.48	2265	13773
T_{12}: Mechanical scarification (2 side)	88.50	27.38	187.38	2423	16583
S. Em±	0.52	0.05	0.05	14	75
CD (p=0.01)	1.48	0.13	0.13	40	214
CV	1.56	0.41	0.07	2	2

Table 4: Effect of dormancy breaking treatments on α –amylase activity (µg g-1) in shankapushpi (*Clitorea ternatea* L.)

Treatments	Shankapushpi
T_{12}	69.21
T_{10}	61.36
T_1	58.38
T_6	56.68
T_0	44.28
S.Em±	0.633
CD	1.92

Treatment details
T_0: Control
T_1: GA_3 (24 hr)
T_6: Soaking in water (48 hr)
T_{10}: Acid scarification (6 minutes)
T_{12}: Mechanical scarification (2 side)

Esterase (EST) isozyme profile as influenced by dormancy breaking treatments in shankapushpi

Banding intensity and relative mobility of esterase (EST) isozyme (plate 1 and Table 5)

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 shankapushpi. The dormancy breaking treatments from 1 to 5 were scored as low, medium, high intensity and very high intensity with Rm value ranged from 0.0158 to 0.0871. All the dormancy breaking treatments showed significant differences either by the presence or absence of bands and as well as their intensity.

Band No. 1: It was composed of single type of band *i.e.,* very high intensity band with an Rm value of 0.000 to 0.0158 present only in T_{10}. It was absent in rest of the treatments.

Band No. 2: It was composed of single type of band *i.e.,* very high intensity band with an Rm value of 0.0158 to 0.0185 present only in T_{12}. It was absent in rest of the treatments.

Band No. 3: It was characterized of single type of band *i.e.,* low intensity band with an Rm value of 0.0185 to 0.0227 present in T_6 and T_1. It was absent in rest of the treatments.

Band No. 4: It was categorized of single type of band *i.e.,* low intensity band with an Rm value of 0.0227 to 0.0 236 present only in T_6. It was absent in rest of the treatments.

Band No. 5: It was characterized of single type of band *i.e.,* high intensity band with an Rm value of 0.0 236 to 0.0298 present only in T_6. It was absent in rest of the treatments.

Band No. 6: It was composed of single type of band *i.e.,* high intensity band with an Rm value of 0.0298 to 0.0331 present only in T_{12}. It was absent in rest of the treatments.

Band No. 7: It was consist of two types of bands based on intensity with Rm value of 0.0331 to 0.0352. Very high intensity band was paramagnetic in T_{10} and high intensity band was noticed in T_1. However it was absent in remaining treatments.

Band No. 8: It was categorized of single type of band *i.e.,* low intensity band with an Rm value of 0.0352 to 0.0511 present in T_6 and T_0. It was absent in rest of the treatments.

Band No. 9: It was composed of single type of band *i.e.,* low intensity band with an Rm value of 0 0.0511 to 0.0564 present in T_6 and T_0. It was absent in remaining treatments.

Band No. 10: It was composed of single type of band *i.e.,* high intensity band with an Rm value of 0.0564 to 0.0631 present in $T_{10,}$ T_{12} and T_1. It was absent in remaining treatments.

Band No. 11: It was consists of single type of band *i.e.,* high intensity band with an Rm value of 0.0631 to 0.0732 present only in T_6. It was absent in remaining treatments.

Band No. 12: It was comprises of single type of band *i.e.,* high intensity band with an Rm value of 0.0732 to 0.0751 present in T_{10} and T_{12}. It was absent in remaining treatments.

Band No. 13: It was consist of two types of bands based on intensity with Rm value of 0.0751 to 0.0767. Very high intensity band was paramagnetic in T_{10} and high intensity band was noticed in T_{12} and T_1. However it was absent in remaining treatments.

Band No. 14: It was differentiated in to two types of bands based on intensity with Rm value of 0.0767 to 0.0792. High intensity band was spotted in T_0 and high intensity band was noticed in T_{10}, T_{12} and T_1. However it was absent in remaining treatments.

Band No. 15: It was Comprises of single type of band *i.e.,* high intensity band with an Rm value of 0.0792 to 0.0813 present in T_{10}, T_{12} and T_0. It was absent in remaining treatments.

Band No. 16: It was composed of single type of band *i.e.,* high intensity band with an Rm value of 0.0813 to 0.0871 present only in T_6. It was absent in rest of the treatments.

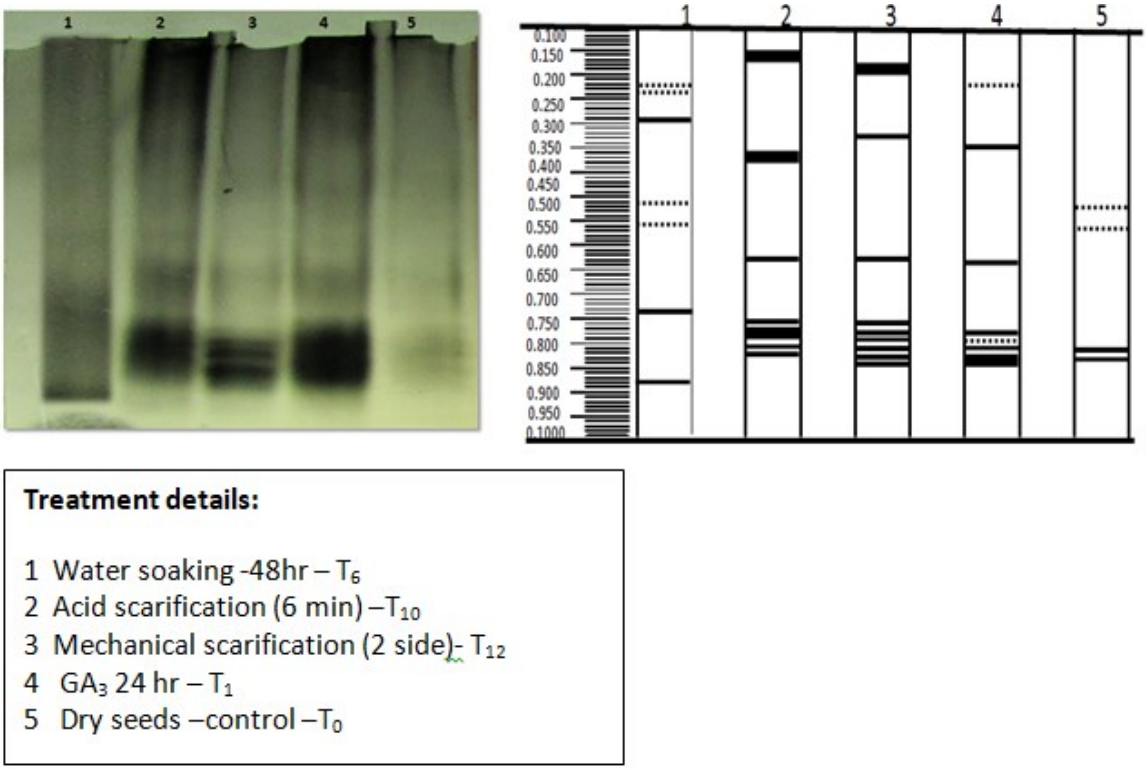

Plate 1: Banding pattern and zymogram of esterase activity in shankapushpi

Table 5: Effect of dormancy breaking treatments on esterase profile of shankapushpi

Band No.	Rm value	1	2	3	4	5
1	0.0158		++++			
2	0.0185			++++		
3	0.0227	+			+	
4	0.0236	+				
5	0.0298	+++				
6	0.0331			+++		
7	0.0352		++++		+++	
8	0.0511	+				+
9	0.0564	+				+
10	0.0631		+++	+++	+++	
11	0.0732	+++				
12	0.0751		+++	+++		
13	0.0767		++++	+++	+++	
14	0.0792		++	++	++	+++
15	0.0813		+++	+++		+++
	0.0871	++				

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

Treatment details

1. Water soaking -48hr – T_6
2. Acid scarification (6 min) –T_{10}
3. Mechanical scarification (2 side)- T_{12}
4. GA_3 24hr – T_1
5. Dry seeds –control –T_0

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 all the thee crops indicate that the pattern of enzyme polymorphism changes during dormancy breaking. These adequately demonstrate that there is qualitative change in enzymes during dormancy breaking.

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