Overcoming seed coat imposed dormancy in wild species of pigeonpea (Cajanus cajan L. Millsp.)

Amrit Lamichaney (amritiipr@gmail.com)
Indian Institute of Pulses Research https://orcid.org/0000-0003-0345-5863
Satheesh Naik SJ
Indian Institute of Pulses Research
Kali Krishna Hazra
Indian Institute of Pulses Research
Dibendu Datta
Indian Institute of Pulses Research
PK Katiyar
Indian Institute of Pulses Research

Research Article

Keywords: Cajanus, Rynchosia, dormancy break, hardseededness, wild accession

Posted Date: January 5th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2408280/v1

Abstract

Occurrence of physical dormancy in the form of hard seed coat prevents proper utilisation and conservation of wild species of pigeonpea, which offers a source of many useful traits. Hence, in the present investigation attempt has been made to understand the variability in hardseededness and the pre-treatments to overcome it in 44 accessions comprising of 10 wild pigeonpea species (*Cajanus scarabaeoides, C. crassus*, *C. platycarpus*, *C. cajanifolius, C. lineatus*, *Rynchosia minima*, *R. bracteata*, *R* rothii, *R rufescens* and *R. aurea*). The level of hardseededness varied from 36–100%, with an average of 76.5%. Likewise, the time to initiate germination (T1), achieve 50% germination (T50) and mean germination time (MGT) varied between 16.18-249.9h, 94.7-607.7h and 37.5-153.2h, respectively. Overall, all the treatments reduced hardseededness and improved germination over the control, with partial incision on seed coat (PSCI) being the best treatment for all the species recording 70–98% germination, except for *R. aurea* (30%) and *C. cajanifolius* (59%). The hot water treatment (HWT) for 30 and 60 minutes was the best treatment for *C. cajanifolius* recording germination of 66–70% as compare to 11% in control. The sulphuric acid scarification (SAT) for 2 m was the best pre-treatment for overcoming dormancy and improving germination in *R. bracteata*, *R. rothii* and *R. rufescens*. The result indicated a significant inter and intra species variation in the effectiveness of pre-treatments in overcoming physical dormancy, suggesting that the optimum pre-treatment for overcoming hardseededness should be standardised for each accession. Also the dormancy breaking pre-treatments not only reduced hardseededness and improved final germination percent but it also reduced time for T1, T50 and MGT, suggesting that use of such pre-treatments before sowing could result into higher, uniform and rapid germination.

Introduction

The genus *Cajanus* belongs to the family Papilionaceae, sub-family Papilionoideae, tribe Phaseoleae and sub-tribe *Cajaninae*. Genus *Cajanus* has 32 different related species, among those the species *cajan* is the only domesticated species (Mallikarjuna et al. 2011). On the evidence of genetic cross-compatibility, 10 Cajanus species namely, *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lanceolatus*, *C. latisepalus*, *C. lineatus*, *C. sericeus*, *C. trinervius*, *C. scarabaeoides* and *C. reticulates* are placed under gene pool 2, and are freely crossable with the cultivated types; therefore, they are often used by the breeders for new trait transfer through pre-breeding programme. The rest of the 22 species, which do not cross with *C. cajan* are placed in the tertiary gene pool (Remanandan 1990). Likewise, there are 11 related generas under sub-tribe *Cajaninae* which are related to the genus *Cajanus* namely, Adenodolichos, Baukea, Carissoa, Dunbaria A., Dunbaria W., Eriosema C., Eriosema D., Flemingia Roxb. Ex Aiton., Reichenb, Rhynchosia Lour., Paracalyx Roxb. Ali, (Mallikarjuna et al. 2011). The wild pigeonpea species exhibits great variability and are an excellent source of plant genetic resources for resistance against biotic/abiotic stresses, plant architecture, improving the nutritional value etc and could be utilised as food, feed, forage, cover crops (Table 1).

Table 1
Detail of the experimental material used in the study, their use and collection site

Species	Collection number	Useful traits	Source of collection
C. scarabaeoides	ICP 15690, ICP 15688, ICP 15699, ICP 15687, ICP 15710, ICP 15712, ICP 15731, ICP 15727, ICP 15886, ICP 15697, ICP 15779, ICP 15797, DD/SPD – 2, DD/SPD-3, SG/DD- 43, SG/DD- 13, SG/DD- 63	Resistance against fusarium wilt, SMD and cyst nematode, early flowering, terminal heat tolerance and pod borer tolerance, source of CMS (A2 cytoplasm), high protein	NBPGR, ICRISAT, Chhattisgarh, West Bengal, Jharkhand and Madhya Pradesh
C. cajanifolius	ICP 15630, ICP 15631, ICP 15632	Salinity and pod borer tolerance, source of CMS (A5 cytoplasm)	NBPGR, ICRISAT
C. lineatus	ICP 15643, SG/DD- 72	Resistance to SMD and alternaria blight, drought tolerance, source of CMS (A6 cytoplasm)	NBPGR, ICRISAT
C. crassus	ICP 15767, ICP 15616, ICP 15771, ICP 15773, ICP 15774, ICP 15776	Source for A3 cytoplasm	NBPGR, ICRISAT
C. platycarpus	ICP 15665, ICP 15661, ICP 15663, ICP 15664, DD/SPD- 7, DD/SPD – 1	Resistance to phytophthora stem blight and alternaria blight, salinity tolerance, annual plant growth, early vigour, photoperiod insensitivity, extra early flowering along with extra early maturity, high protein content	NBPGR, ICRISAT, Chhattisgarh, West Bengal, Jharkhand and Madhya Pradesh
Rhynchosia minima	ICP 15838, SG/DD- 2, SG/DD- 3	Medicinal value and forage	NBPGR, Chhattisgarh, West Bengal, Jharkhand and Madhya Pradesh
R. bracteata	ICP 15817, ICP 15815, ICP 15817	Tolerance to pod borer and pod fly	NBPGR, ICRISAT
R. rothii	ICP 15851, ICP 15856	Early flowering, photoperiod insensitivity, High seed protein content	NBPGR, ICRISAT
R. rufescens	ICP 15861	Leaves contain flavones and flavone glycosides	NBPGR, ICRISAT
R. aurea	ICP 15809		NBPGR

The inability of the seed to germinate under optimal condition is termed as seed dormancy. Seed dormancy is considered undesirable in crop species as it causes non-uniform and delayed germination/emergence and affects yield, prolongs cooking time and hampers the seed testing protocols. However, in wild species, dormancy is considered as a survival trait, as it reduces the chance of species extinction if unfavourable condition kills all the readily germinated seeds (Baskin and Baskin 2014). Numerous studies reports occurrence of seed dormancy in wild relatives of a crop species (Lamichaney et al. 2018; Singh et al. 2020; Lamichaney et al. 2021). The wild species of pigeonpea are a great source of numerous traits but for its efficient utilization, successful germination is a pre-requisite. In legumes, like pigeonpea, physical dormancy in the form of hard seededness is the most prevalent type of seed dormancy (Singh et al. 2020; Cavallaro et al. 2021; Lamichaney et al. 2022). The hard seed prevents entry of water into the seed, thereby restricts the metabolic function essential for germination to occur. The lack of

information on overcoming hardseededness of these wild accessions creates a problem and is a bottleneck during its conservation and utilization. Therefore, an efficient and effective technique for removing hard seededness of these species must be developed as a considerable inter and intra specific variation for seed dormancy exists (Mira et al. 2017; Singh et al. 2020).

The seed coat of leguminoseae constitutes of a single palisade layer of packed and elongated sclereids and non-uniformly thickened cell wall. Cuticle or waxy deposition, lignin, tannin, lipids, phenolics, callose, suberin, pigmentation etc are associated with hardseededness in legumes (Smykal et al. 2014; Lamichaney et al. 2017; Cavallaro et al. 2021). An earlier report claims some physical, chemical, mechanical methods for removing dormancy imposed by seed coat impermeability. Among these, most widely used method to break hardseededness are mechanical abrasion, sulphuric acid scarification, hot water/air treatment, exposing seeds to fluctuating temperatures, drying, ultrasonic treatment (Rifna et al. 2019; Singh et al. 2020; Baskin et al. 2004; Keshtkar et al. 2010; Abbasi 2020). A comprehensive analysis of germination, hardseededness and methods overcoming the coat imposed dormancy is lacking in the wild species of pigeonpea. Therefore, the present study was carried out to understand the level of hardseededness, intra/inter specific variability and methods to overcome hardseededness and enhance germination in 44 wild accessions of pigeonpea belonging to 10 different species.

Materials And Methods

The freshly harvested seeds of 44 accessions of 10 wild species (17 accessions of *Cajanus scarabaeoides*, 6 each of *C. crassus* and *C. platycarpus*, 3 each of *C. cajanifolius, Rynchosia minima*, *R. bracteata*, 2 each of *R rothii* and *C. lineatus* and 1 each of *R rufescens* and *R. aurea) w*ere used in this experiment (Fig. 1) to study the occurrence of hard seededness and ways to overcome it. Seeds of these accessions were subjected to nine dormancy breaking treatments, namely, control, hot air treatment for 3, 5, 7, 14 days, hot water treatment for 30, 60 minutes, concentrated sulphuric acid scarification for 2, 5 minutes and partial incision on the distal end of seed coat. The details of the dormancy breaking treatments are presented in Table 2. The seeds were placed in a muslin cloth bag and were placed in an oven maintained at (45^{o}C) and hot water bath maintained at 50^{o}C for hot air and hot water treatment. After hot water treatment, the seeds were rinsed in running tap water and surfaced dried. For, acid scarification, the seeds were immersed in a concentrated sulphuric acid for 2 and 5 minutes. After acid scarification, the seeds were thoroughly washed in running tap water for 2–3 minutes and allowed to surface dry. For partial incision of seed coat, at the distal end a small incision was made using a needle and a sharp blade. Dry normal seed was used as a control to check the efficiency of different dormancy braking treatments.

Table 2
Description of pre-treatments used for overcoming hardseededness

S.No.	Dormancy breaking treatments	Duration	Abbreviation
1	Control	-	CT
2	Hot air treatment (45^{o}C)	3 days	HAT_{3D}
3		5 days	HAT_{5D}
4		7 days	HAT_{7D}
5		14 days	HAT_{14D}
6	Concentrated sulphuric acid scarification	2 minutes	SA_{2M}
7		5 minutes	SA_{5M}
8	Hot water treatment (50^{o}C)	30 minutes	HWT_{30M}
9		60 minutes	HWT_{60M}
10	Partial incision of seed coat	-	PSCI

After each treatment along with control, 25 seeds of each accession in three replications were placed in plastic Petri dishes of 11 cm diameter, lined with 2 water soaked Whatman No. 1 filter paper. As per International Seed Testing Association (ISTA 2022), the optimum temperature for testing germination of *Cajanus cajan* is either 25^{o}C constant or alternate 20/30 ^{o}C. Therefore, the seeds of all the tested species were incubated at a constant temperature of 25^{o}C, since information on the germination testing protocol for these species are not available. Daily observation on number of germinated, hard, and dead seeds was taken for 15 days. The seeds were considered germinated when radicle has attained an approximate length of 2mm. Those seeds which failed to imbibe water were counted as hard seeds. The seeds which have imbibed water but not germinated and are attacked by fungus are considered as dead seeds.

At the end of the experiment, the final percentage germination, actual time to 1% germination or time taken to initiate germination (T1), actual time to 50% germination (T50), and mean germination time were calculated. The daily germination performance were interpreted by “Germinator curve-fitting1.29.xls” microsoft excel model” (Joosen et al. 2010) using the following equation (El-Kassaby et al. 2008).

$$Y = Yo + \frac{aX^b}{C^b + X^b}$$

Where, y is cumulative germination percentage at time x, y0 is intercept on the y axis, a is maximum germination percent, b is steepness of the curve and c is time taken by 50% of seeds to germinate (T50). The arcsine transformed germination data, T1, T50, and MGT data were statistically analysed by completely randomized one-way ANOVA using online software OP-STAT (Sheoran et al. 1988).

Results And Discussion

Significant inter/intra species variation was observed for hardseededness, time to initiate germination (T1), time to achieve 50% germination (T50), and mean germination time (MGT) in 44 accessions representing 10 wild species of pigeonpea. The level of hardseededness varied from 36–100%, with an average of 76.5%. Very few seeds that germinated were the non dormant seeds in a seed lot that could imbibe water and initiate the metabolic processes for germination. Likewise, the T1, T50 and MGT varied between 16.18-249.9h, 94.7-607.7h and 37.5-153.2h, respectively (Fig. 2). In general, there was a significant proportion of hardseeds in the accessions tested, therefore, in the present investigation an attempt was made to improve final germination, germination speed using different techniques that would facilitate its utilization and conservation. The germination of dormant seeds is poor, slow and not uniform, therefore it is necessary to use treatments that can promote high, rapid and uniform germination (Delachiave and Depinho 2003).

Overall, all the treatments reduced hardseededness and improved germination over the control, with PSCI being the best treatment for all the species recording 70–98% germination, except for *R aurea* (30%) and *C cajanifolius* (59%). The HWT for 30 and 60 minutes was the best treatment for C cajanifolius recording germination of 66–70% as compare to 11% in control. However, the response of rest of the species to HWT was variable, and not much improvement was observed in C scarabaeoides, R bracteata, R minima, R aurea and C platycarpus. The sulphuric acid scarification (SAT) for 2 m was the best pre-treatment for overcoming dormancy and improving germination in R bracteata (along with PSCI & HAT3D), R rothii and R rufescens (along with PSCI). The pre-treating seeds with SAT for 2 m did not improve germination of R minima and C platycarpus, however, reduced germination in R aurea. Likewise, SAT for 5 minutes improved germination in all the species except for R minima and R bracteata. Overall, a hot air treatment (HAT) for longer time were more effective in improving germination than for short duration, but was absolutely ineffective for C platycarpus (Fig. 3). The result indicated a significant inter and intra species variation in the effectiveness of pre-treatments in overcoming physical dormancy, suggesting that the optimum pre-treatment for overcoming hardseededness should be standardised for each accession. The result on inter/intra species variation on efficiency of of pre-treatments in overcoming hard seeds is further confirmed in PCA scatter plot (Fig. 4). In accordance to our result Singh et al (2020) and Ellis et al (1985) inferred that single pre-treatment could not be effective in removing dormancy for all accessions. The inter/intra species variation in the effectiveness of pre-treatments in overcoming hardseeds may be attributed to the testa properties like structure and composition of palisade layer which are determined by genotype and production/storage environment (Tomar and Kumari 1991).

The time course of seed germination of 10 species with different pre-treatments revealed that PSCI resulted into faster germination in all the species (Fig. 5). In control, the maximum and minimum average time taken to initiate germination was recorded in C platycarpus (78.20 h) and C cajanifolius (35.27 h) with an average of 60.46 h. Interestingly, all the HAT and HWT60m resulted into delayed initiation of germination as compared to control. The PSCI pre-treatment resulted into significant reduction in germination initiation time from 60.46 h (control) to 25.70 h (Fig. 6). In control seeds, only 8 out of 44 accessions recorded 50% germination the time of which varied from 94.4 h (ICP 15856) to 607.7 h (SG/DD 43). The HAT for 3, 5, 7 and 14 days resulted into ≥ 50% germination in 10, 10, 28 and 20 accessions suggesting the effectiveness of these treatments in removing hardseeds and improving germination percentage. Likewise, SAT for 2 and 5 m resulted into ≥ 50% germination in 24 and 21 accessions, with T50 varying from 40.4h (ICP 15643) – 357.1h (ICP 15774) and 47.5h (ICP 15688) – 385.2h (ICP 15817), respectively. The HWT for 30 and 60 m caused ≥ 50% germination in 20 and 15 accessions. Notably PSCI resulted into ≥ 50% germination in 40 out of 44 accessions and T50 varied from 41.9h (ICP 15817) to 457.7 h (ICP 15661). All the pre-treatments failed to increase the germination of 6 accessions of C platycarpus ≥ 50%, except for SAT and PSCI. However, none of the pre-treatments were effective in improving germination of R aurea (ICP 15809) by ≥ 50% (Table 3). The faster germination as recorded after PSCI inferred that the embryo is ready to germinate but the hard

water impermeable seed coat hampers its germination. The effectiveness of pre-treatments in terms of MGT was similar as that for T1 and T50, whereby the PSCI resulted into faster germination as compared to other treatments. The entire HAT recorded significantly higher MGT as compared to control (Fig. 7). The hardness of the seed coat, attributed by the palisade layer (PL) of packed and elongated sclereids and non-uniformly thickened cell wall is responsible for seed coat imposed impermeability. Also the water impermeable compounds located in the outside of PL attributes to hardseeds in legumes (Van Staden et al. 1989). Cuticle or waxy deposition, lignin, tannin, lipids, phenolics, callose, suberin, pigmentation etc are responsible for water impermeability in legume seeds (Ma et al. 2004; Smykal et al. 2014; Cavallaro et al. 2021).

Table 3
Effect of different dormancy overcoming pre-treatments on time to achieve 50% germination (T50)

Accessions	CT	HAT_{3D}	HAT_{5D}	HAT_{7D}	HAT_{14D}	ACT_{2M}	ACT_{5M}	HWT_{30M}	HWT_{60M}	PSCI
ICP 15690	366.7	-	-	-	-	-	-	162.2	-	47.3
ICP 15688	233.8	219.5	262.6	194.9	149.6	61.7	47.5	184.8	-	46.6
DD/SPD – 2	-	-	-	309.6	-	49.1	95.5	-	-	43.3
ICP 15699	-	-	-	276.5	390.6	-	-	-	-	42.8
ICP 15687	-	-	-	221.9	476.3	-	98.8	316.3	-	56.7
ICP 15710	-	-	-	351.5	-	-	56.3	-	-	54.7
ICP 15712	-	-	-	227.4	-	122.9	203.7	-	-	63.0
DD/SPD-3	-	-	-	227.5	601.5	-	166.0	80.1	-	44.5
ICP 15731	-	427.0	-	319.6	281.9	66.7	-	-	-	53.1
ICP 15727	-	-	-	222.4	208.4	55.8	117.6	173.9	636.1	56.5
SG/DD- 43	607.7	-	-	197.1	231.5	50.0	325.5	100.8	-	45.6
SG/DD- 13	-	-	-	-	-	86.5	-	-	-	44.9
ICP 15886	-	-	-	575.2	-	177.6	-	-	-	50.8
ICP 15697	-	-	-	595.4	-	109.2	-	-	-	67.4
ICP 15779	-	-	-	1239.9	-	187.6	-	161.6	-	-
ICP 15797	-	-	-	343.0	-	-	-	307.8	-	44.4
SG/DD- 63	229.0	-	-	-	473.3	164.8	-	135.1	166.2	-
ICP 15767	-	-	-	70.3	-	53.7	62.9	45.6	41.4	44.4
ICP 15616	-	-	-	-	-	55.8	-	-	39.9	42.1
ICP 15771	-	-	-	83.1	90.4	48.1	-	-	43.7	49.0
ICP 15773	-	85.8	-	69.7	70.8	45.1	52.4	50.3	51.3	71.6
ICP 15774	-	-	-	108.4	74.0	357.1	261.2	39.0	78.0	42.1
ICP 15776	-	91.1	99.7	87.1	81.1	82.5	281.7	65.8	46.2	44.4
ICP 15630	-	47.2	46.9	55.8	-	-	69.4	50.6	24.7	45.6
ICP 15631	-	-	-	76.1	122.2	-	-	-	44.6	-
ICP 15632	-	-	-	-	-	-	70.2	175.6	-	44.4
SG/DD- 2	-	-	-	-	-	-	-	-	-	44.4
SG/DD- 3	-	-	284.2	279.3	343.3	-	-	-	-	45.5
ICP 15838	155.0	61.0	83.3	84.1	48.3	-	-	53.3	-	44.4

Accessions	CT	HAT_{3D}	HAT_{5D}	HAT_{7D}	HAT_{14D}	ACT_{2M}	ACT_{5M}	HWT_{30M}	HWT_{60M}	PSCI
ICP 15817	181.5	220.8	226.5	221.5	-	95.1	385.2	-	-	76.4
ICP 15815	101.2	170.9	176.6	236.5	184.5	45.5	-	89.4	351.6	74.5
ICP 15817	-	163.8	185.5	143.4	174.6	124.2	77.2	63.1	56.5	41.9
ICP 15851	-	-	-	-	326.7	59.8	115.8	159.3	113.5	46.6
ICP 15856	94.4	131.1	83.8	116.9	-	169.1	-	-	53.8	76.7
ICP 15861	-	-	339.3	205.7	297.2	50.3	67.9	-	-	49.8
ICP 15809	-	-	-	-	-	-	-	-	-	-
ICP 15643	-	-	-	-	101.1	40.4	50.8	140.6	234.8	79.9
SG/DD- 72	-	-	-	-	-	-	-	-	-	45.4
DD/SPD- 7	-	-	-	-	-	-	-	-	-	50.8
ICP 15665	-	-	-	-	-	-	120.5	-	-	49.3
DD/SPD – 1	-	-	-	-	-	-	-	-	-	47.0
ICP 15661	-	-	-	-	-	-	-	-	-	87.7
ICP 15663	-	-	-	-	-	-	56.4	-	-	47.5
ICP 15664	-	-	-	-	-	-	-	-	-	42.3

In the present investigation, PSCI was the most effective pre-treatment that not only increased final seed germination but also reduced the time to initiate germination (T1), time to achieve 50% germination (T50%) and mean germination time (MGT) as compared to control. The small incision made in the distal end of the seeds facilitated entry of water and initiate the metabolic processes for germination resulting into reduced hardseeds, improved germination and germination time. Several study reports different pre-treatments effective in removing hard seeds in legumes. Sulphuric acid scarification is reported to be best treatment in overcoming hard seeds and improving germination in carob (*Ceratonia siliqua* L.) (Cavallaro et al. 2021), *Vigna* spp. (Wang et al. 2007), *Onobrychis* species (Majidi and Barati 2011). The SAT performed fairly well in the accessions tested and was the best treatment for overcoming dormancy and improving germination in R bracteata, R rothii and R rufescens. However, the duration of acid scarification should be well standardized or else could cause embryo damage and even kill the seed (Martin and Cuadra 2004; Silveira and Fernandes 2006). Whereas, mechanical scarification was the best for promoting germination in *Vigna species* and *Trifolium species* (Singh et al. 2020; Abbasi 2020). Freeze-thaw scarification was reported to improved germination in *Lupinus albus* and *Trifolium pratense* by overcoming coat imposed dormancy (Tiryaki and Topu 2014).

The wild relatives are very precious and needs to be conserved. During conservation of these germplasm, accurate estimation of the viability is important, while during their utilization it is vital to ensure timely and uniform germination. Therefore, the pre-treatments identified in this study could be utilised for conservation and utilization of the wild accessions of pigeonpea where physical dormancy in the form of hard seed coat is prevalent.

Declarations

Acknowledgements Authors are grateful to the ICAR-Indian Institute of Pulses Research, Kanpur for providing support and facilities to carry out this work.

Conflict of interest The authors declare no conflict of interest.

References

1. Abbasi MR (2020) Breaking seed dormancy in some Iranian wild clovers (*Trifolium spp.*) selected as promising new germplasm. Genet Resour Crop Evol 67(6):1577–1585
2. Baskin C, Baskin JM (2014) Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, p 1600.
3. Baskin JM, Davis BH, Baskin CC, Gleason SM, Cordell S (2004) Physical dormancy in seeds of *Dodonaea viscose* (Sapindales, Sapindaceae) from Hawaii. Seed Sci Res 14(01):81–90
4. Cavallaro V, Maucieri C, Patane C, Fascella G, Pellegrino A, Barbera AC (2021) Polyphenols leaching and seed dormancy in carob (*Ceratonia siliqua* L.) in relation to hot water treatment. Acta Physiologiae Plantarum 43:141
5. Delachiave MEA, Depinho SZ (2003) Scarification, temperature and light in germination of *Senna occidentalis* seed (Caesalpinaceae). Seed Science and Technology 31:225–230
6. El-Kassaby YA, Moss I, Kolotelo D, Stoehr M (2008) Seed germination: mathematical representation and parameters extraction. For Sci 54:220–227
7. Ellis RH, Hong TD, Roberts EH (1985) Handbook of seed technology for genebanks, vol I, Principles and methodology, International Board of Plant Genetic Resources, Rome, Italy
8. International Seed Testing Association (2022) International rules for seed testing. Basserdorf, Switzerland
9. Joosen RVL, Kodde J, Willems LAJ, Ligterink W, Linus H, Hilhorst HWM (2010) GERMINATOR: a software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. Plant J 62:1–12
10. Keshtkar A, Keshtkar H, Razavi S, Dalfardi S (2010) Methods to break seed dormancy of Astragalus cyclophyllon. Afr J Biotechnol 7(21):3874–3877
11. Lamichaney A,Katiyar PK, Laxmi V, PratapA (2018) Variation in pre-harvest sprouting tolerance and fresh seed germination in mungbean (*Vigna radiata* L.) genotypes. Plant Genetic Resources 16(5):437–445
12. Lamichaney A, Kudekallu S, Kamble U, Sarangapany N, Katiyar PK, Bohra A (2017) Differences in seed vigour traits between desi (pigmented) and kabuli (non-pigmented) ecotypes of chickpea (*Cicer arietinum* L.) and its association with field emergence. Journal of Environmental Biology 38(5):735–742
13. Lamichaney A, Pratap A, Katiyar PK, Singh NP (2021) Genetic variability studies and identification of pre-harvest sprouting tolerant wild Vigna. Indian Journal of Agricultural Sciences 91(3):335–339
14. Lamichaney A, Tewari K, Katiyar PK, Parihar AK, Pratap A, Singh F (2022) Implications of exposing mungbean (*Vigna radiata* L.) plant to higher CO_2 concentration on seed quality. International Journal of Biometeorology 10.1007/s00484-022-02366-3
15. Majidi MM, Barati M (2011) Methods for breaking seed dormancy in one cultivated and two wild *Onobrychis* species. Seed Sci Technol 39:44–53
16. Mallikarjuna N, Saxena KB, Laxmi J, Varshney R, Srikanth S, Jadhav D (2012) Differences between *Cajanus cajan* and *C. cajanifolius*, the progenitor species of pigeonpea. Genet Resour Crop Evol 59:411–417
17. Martin I, Cuadra C (2004) Evaluation of different scarification methods to remove hardseededness in *Trifolium subterraneum* and *Medicago polymorpha* accessions of the Spanish base genebank. Seed Sci Technol 32:671–

681

18. Van Staden J, Manning JC, Kelly KM (1989) Legume seeds – the structure: function equation. In: Stirton CH and Zarucchi JL (eds) Advances in legume biology. Monogr Syst Bot Missouri Bot Garden 29:417–450
19. Mira S, Schnadelbach A, Correa EC, Perez-Garcia F, Gonzalez-Benito ME (2017) Variability of physical dormancy in relation to seed mechanical properties of three legume species. Seed Sci Technol 45:540–556
20. Rifna E, Ramanan KR, Mahendran R (2019) Emerging technology applications for improving seed germination. Trends Food Sci Technol 86:95–108
21. Ma F, Cholewa E, Mohamed T, Peterson CA, Gijzen M (2004) Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. Ann Bot 94:213–228
22. Remanandan P (1990) Pigeonpea: genetic resources. In: Nene YL, Hall SD, Sheila VK (eds) The Pigeonpea. CAB International, Wallingford, UK, pp 89–115
23. Singh N, Gore PG, Aravind J (2020) Breaking seed coat impermeability to aid conservation and utilization of wild Vigna species. Genet Resour Crop Evol 67(2):523–529.
24. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS (1998) Statistical software package for agricultural research workers. In: Hooda DS, Hasija RC (eds) Advances in Information Theory, Statistics & Computer Applications, Department of Mathematics Statistics, CCS HAU, Hisar, pp 139–143
25. Silveira FAO, Fernandes GW (2006) Effect of light, temperature and scarification on the germination of *Mimosa foliolosa* (Leguminosae) seeds. Seed Sci Technol 34:585–592
26. Smykal P, Vernoud V, Blair MW, Soukup A, Thompson RD (2014) The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science 5:351
27. Tiryaki I, Topu M (2014) A novel method to overcome coat-imposed seed dormancy in *Lupinus albus* L. and *Trifolium pratense* L. J Bot 647469
28. Tomar RPS, Kumari P (1991) Hard seed studies in black gram. Seed Sci Technol 19:51–56
29. Wang YR, Hanson J, Mariam YW (2007) Effect of sulphuric acid pretreatment on breaking hard seed dormancy in diverse accessions of five wild Vigna species. Seed Sci Technol 35:550–559

Figures

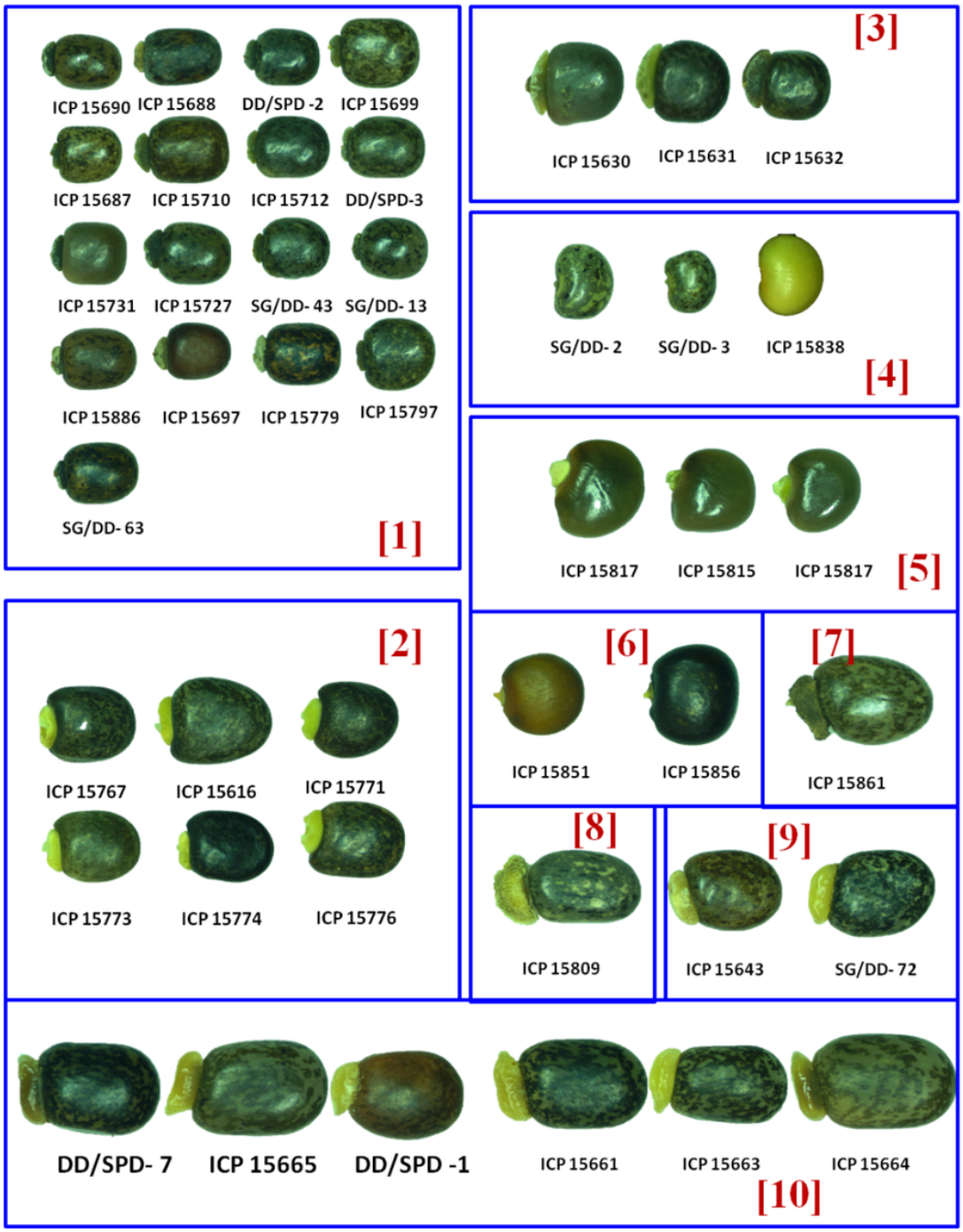

Figure 1

Seeds of forty-four accessions used in the experiment. [1] *C. scarabaeoides,* [2] *C. crassus,* [3] *C. cajanifolius,* [4] *R. minima*, [5] *R. bracteata*, [6] *R. rothii*, [7] *R. rufescens*, [8] *R. aurea*, [9] *C. lineatus*, [10] *C. platycarpus*

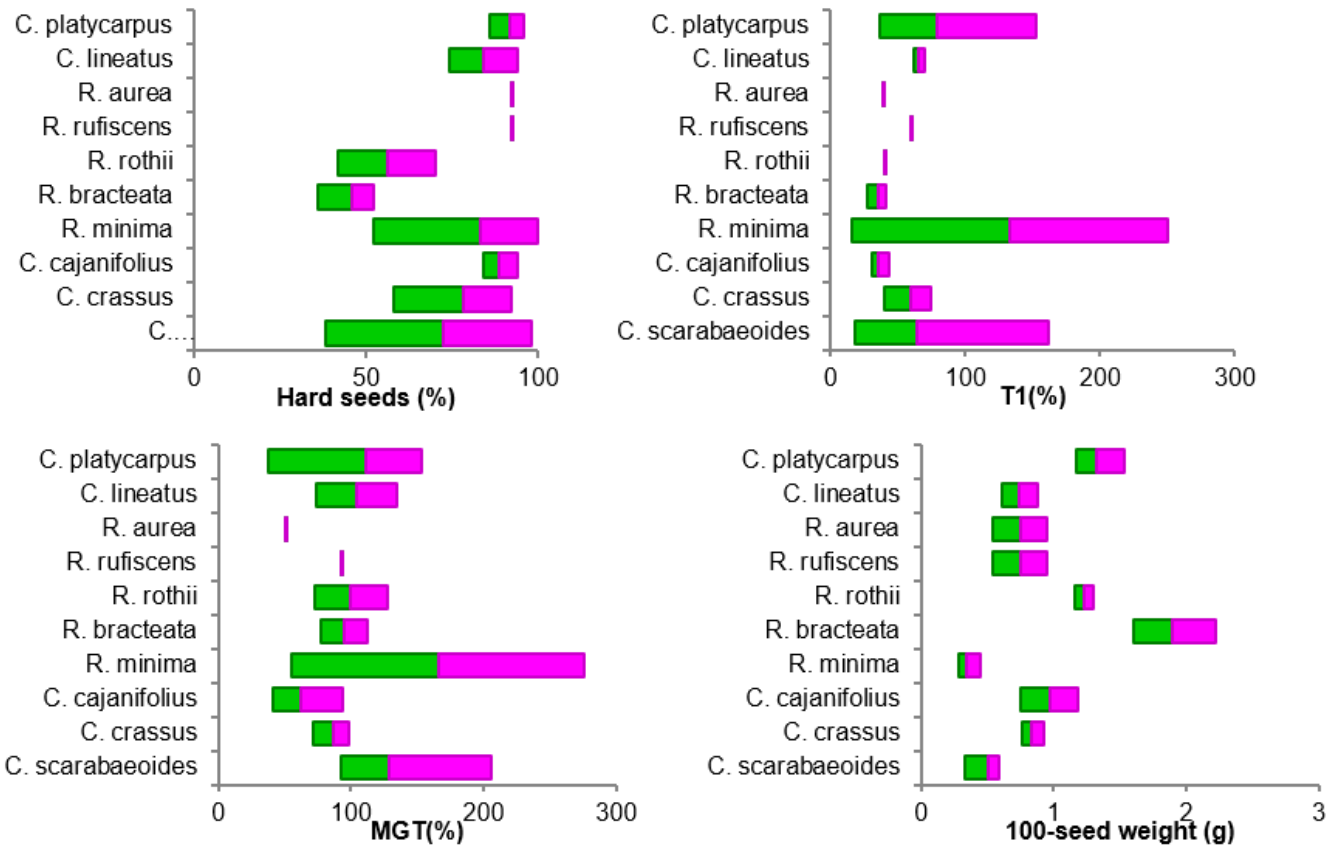

Figure 2

Variation in percent hard seeds, time to initiate germination (T1), mean germination time (MGT) and 100-seed weight in 44 accessions belonging to 10 species.

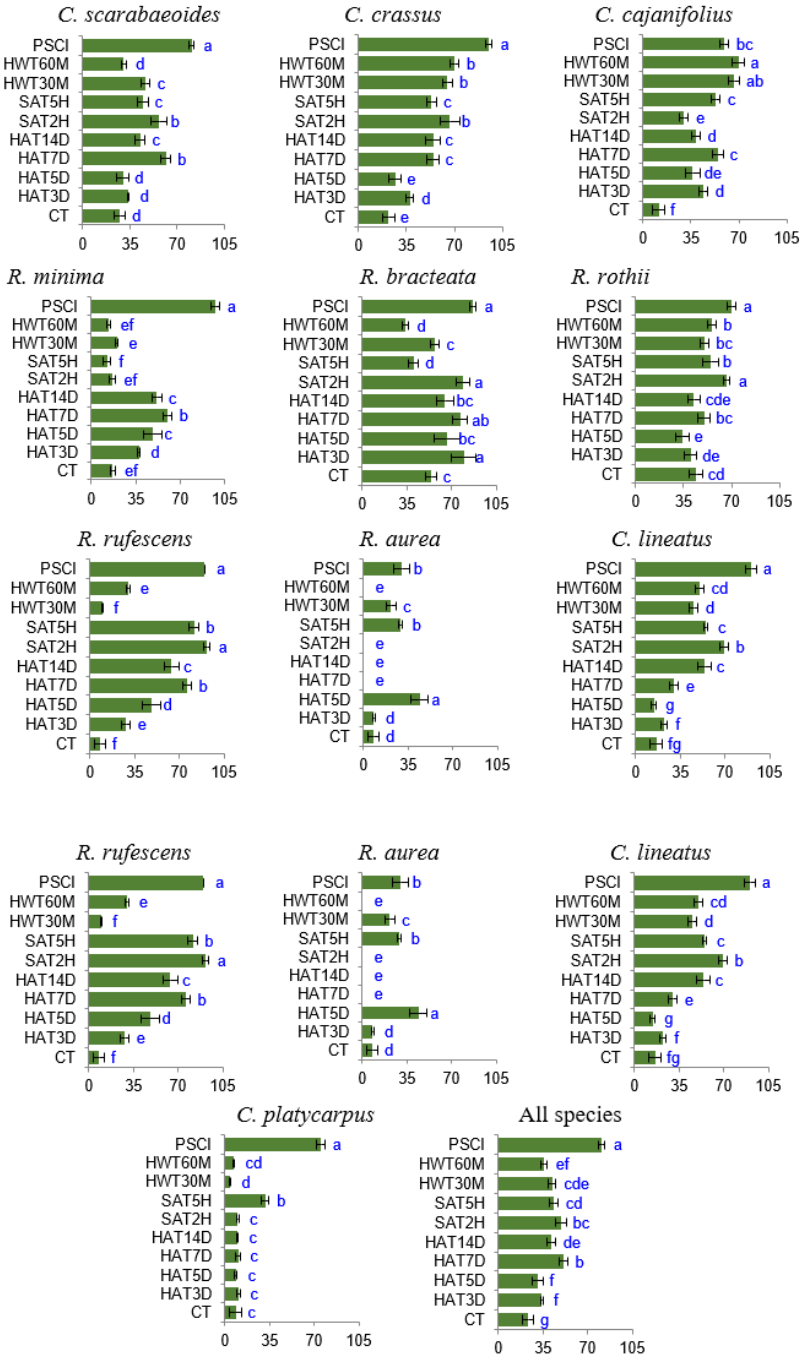

Figure 3

Effect of different dormancy overcoming pre-treatment on seed germination (%) of different wild species of pigeonpea. Error bar represents standard error of means. *a-g* different lowercase letters corresponding to treatment bars are significantly different at $p < 0.05$. data were subjected to arcsine transformation before statistical analysis.

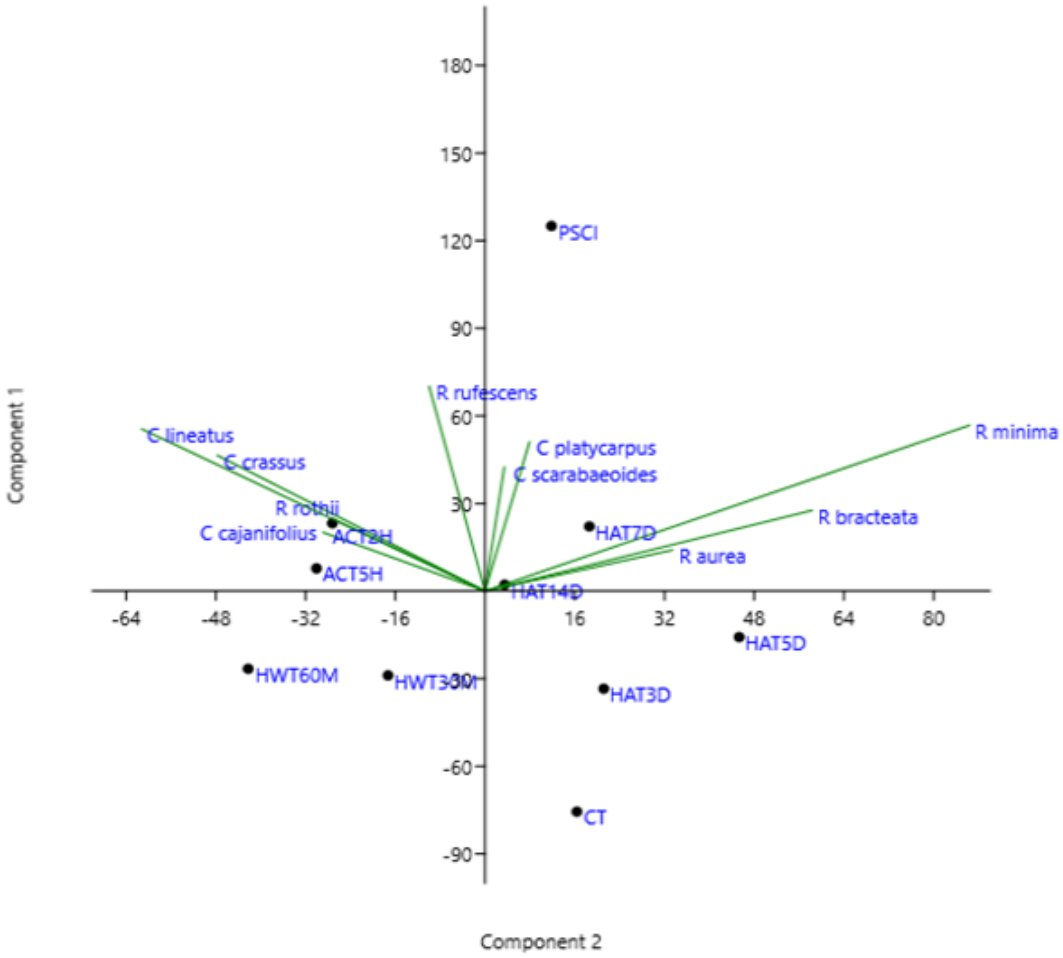

Figure 4

Scatter plot representing the relationship of dormancy overcoming pre-treatments in different species

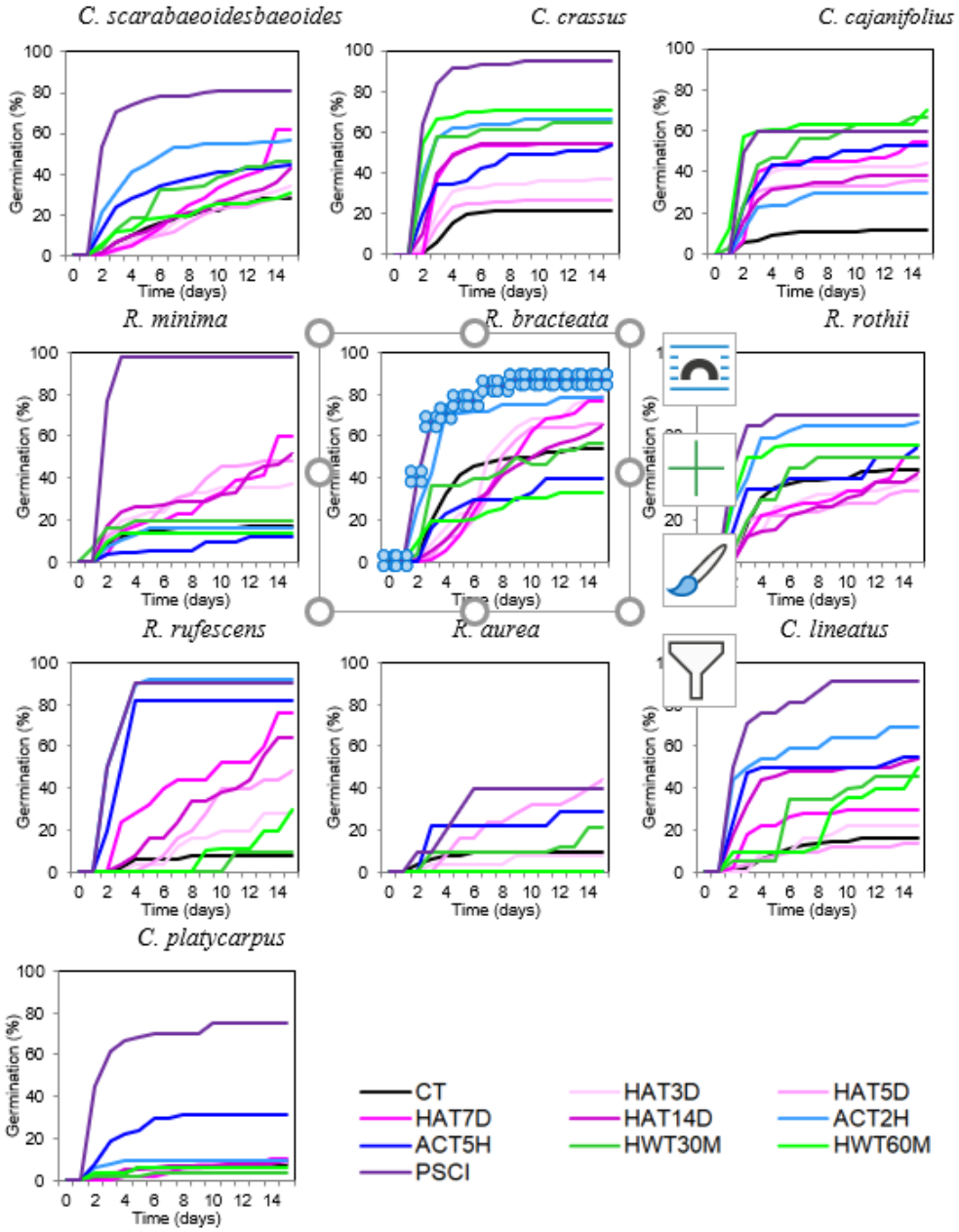

Figure 5

Time course of germination of 10 species of wild pigeonpea after dormancy overcoming pre-tretaments

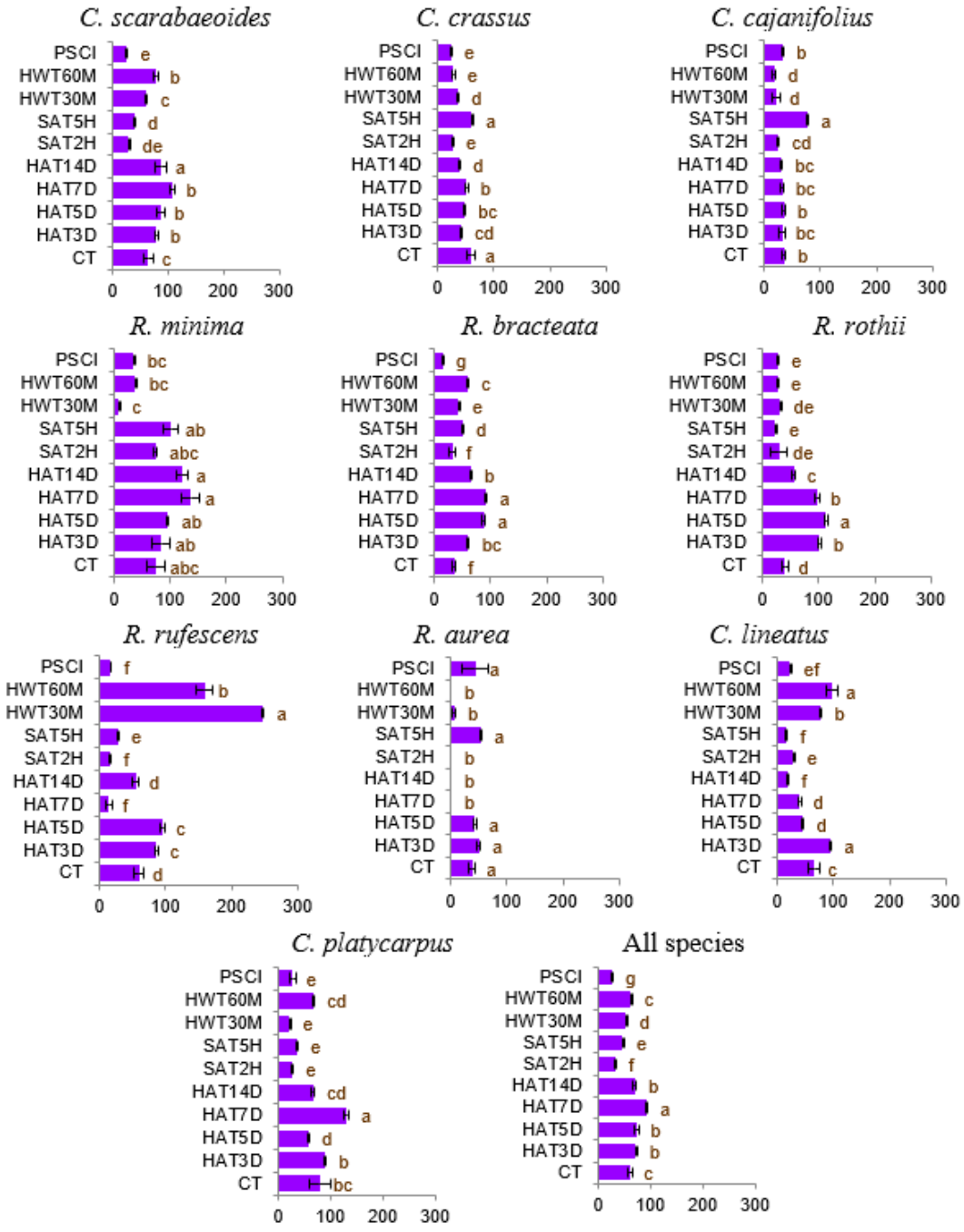

Figure 6

Effect of different dormancy overcoming pre-treatment on time to initiate germination (T1) of different wild species of pigeonpea. Error bar represents standard error of means. *a-g* different lowercase letters corresponding to treatment bars are significantly different at $p < 0.05$. data were subjected to arcsine transformation before statistical analysis.

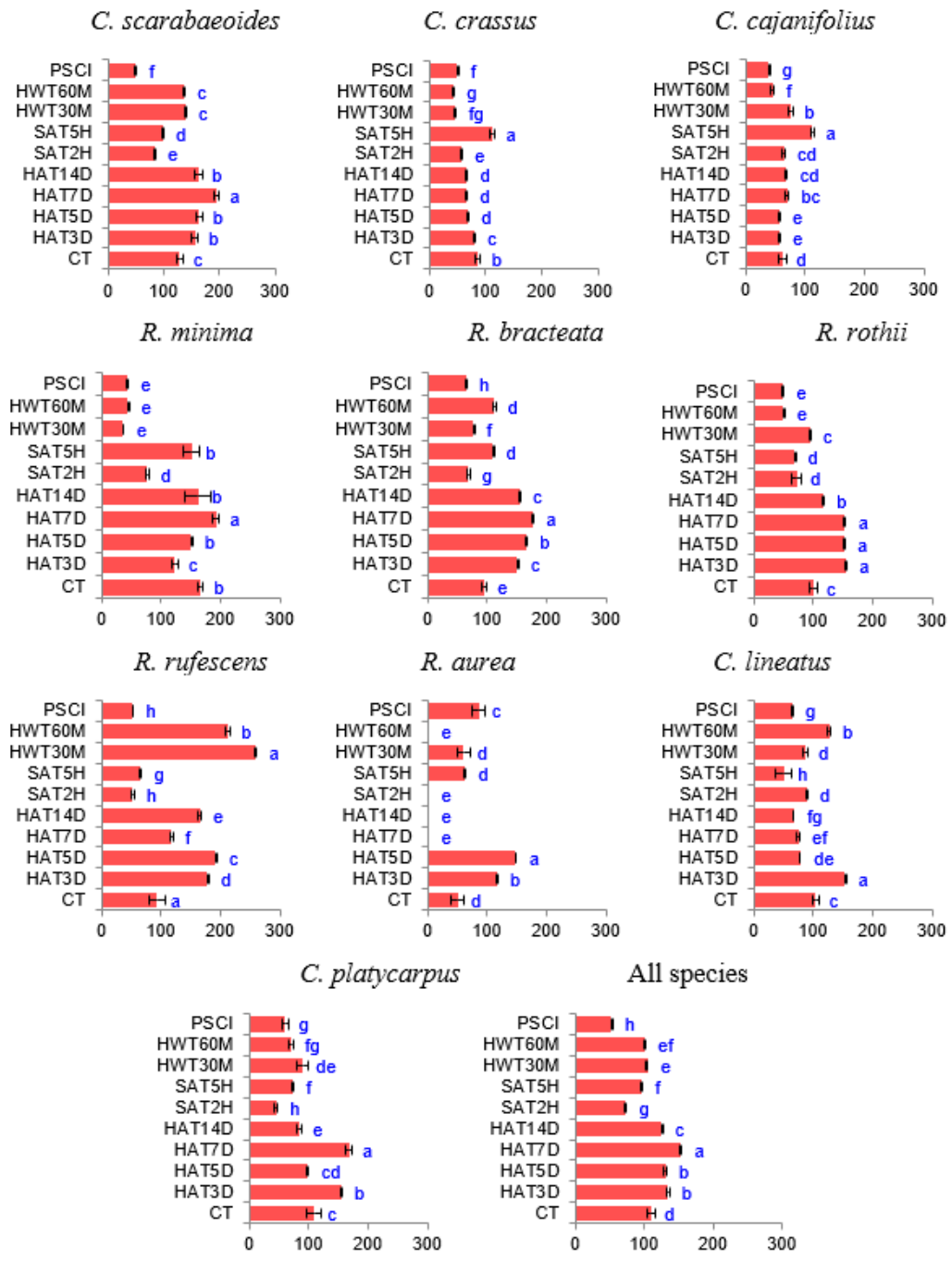

Figure 7

Effect of different dormancy overcoming pre-treatment on mean germination time (MGT) of different wild species of pigeonpea. Error bar represents standard error of means. *a-g* different lowercase letters corresponding to treatment bars are significantly different at $p < 0.05$. data were subjected to arcsine transformation before statistical analysis.