

## Seed Maturation and Hard Seed Development in *Crotalaria* (*Crotalaria juncia*)

M. KHANNA, A.K. VARI, S. BARANWAL, S.P. SHARMA AND I. JETHANI

Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi 110 012  
alicevari@yahoo.com

**ABSTRACT** Field experiments were conducted during *Kharif* 2002 and 2003 to study seed quality in relation to seed development and maturity in four genotypes of *Crotalaria*. The results indicated differences among genotypes in number of days to initiation of flowering and duration of seed maturation. Physiological maturity was attained between 40-50 DAA and at this stage the seeds had maximum germination, vigour and viability. Field maturity was attained at 50-70 DAA, depending upon genotypes and was also influenced by the temperature and humidity prevailed during seed maturation and development. Development of physical seed dormancy (hard seeds) due to impermeable seed coat was found to be closely associated with weather conditions during later stages of seed maturation. Maximum number of hard seeds was observed in the year 2002 probably due to drought like conditions causing a sudden drop in seed moisture at physiological maturity, while in 2003 comparatively milder temperature and higher R.H. prolonged seed maturation process and significantly decreased the hard seed development.

**Key words:** *Crotalaria*, physiological maturity, germination, hard seed

Physiological maturity and harvesting time is of major considerations in the production of quality seeds. At physiological maturity the seed quality attributes are at their highest level. The optimum time of harvest varies for genotype to genotype and it some time creates problem to decide the proper stage of seed harvest. Therefore, identification of the morphological indices associated with physiological and harvest maturity is an important field of investigation. Climatic conditions during flowering, seed development and maturation also have great impact on seed quality attributes like seed size, colour, germination and vigor. Seed moisture content at different harvest occasions also influences the hard seededness in some of the leguminous species [1]. No systematic studies on morphological indices of physiological maturity have been reported for *Crotalaria* species. The present investigation was aimed at studies on the physical and physiological changes in developing seeds and the development of seed dormancy in four cultivars of *Crotalaria juncia*.

### MATERIALS AND METHODS

The experiment was conducted at IARI, New Delhi, using four cultivars of *Crotalaria* viz. Crota L-1, Crota H-1 (early type), Crota ND-1 and Crota Pant-1 (late type) in *Kharif* 2002 and 2003. The sowing was done in the second fortnight of July in 2002 and last week of August in 2003 and all normal cultural practices were followed in raising the crop. The plants were spaced at 60 x 15cm distance in 5m x 4 rows with four replications. *Crotalaria* has indeterminate flowering and under Delhi conditions it lasts for 15-20 days. Differences were there among genotypes regarding the onset of reproductive stage for the four genotypes studied. For seed development study a minimum of fifty flowers each for respective genotypes were tagged daily for about 12 days at uniform stage i.e. when the stigma starts protruding out of keel petal. The pods were harvested in required numbers, starting from 20<sup>th</sup> up to 70<sup>th</sup> days after anthesis (DAA) at 5 days interval. The stages of pod collection were

designated as H<sub>1</sub>-H<sub>11</sub> to represent the 20<sup>th</sup> to 70<sup>th</sup> day of pod development. Following observations were made on the fresh pods such as length of pods, number of seeds/pod, fresh weight of seeds, 100 seed weight, pod and seed coat colour. Four replications of hundred seeds each were used for germination test after surface sterilization of the seeds with 0.1 per cent mercuric chloride for 30 seconds, followed by thorough washings with distilled water. The seeds were germinated using between paper (BP) method at 25°C in an incubator. Seeds were considered germinated when the radicle emerged out from seed coat. The following observations were made on the 6<sup>th</sup> day of germination i.e. percentage germination (excluding hard seeds), root and shoot length and seedling dry weight. At each sampling stage, the moisture content of seeds was estimated by oven method [2].

## RESULTS AND DISCUSSION

Seed maturation refers to morphological, physiological and functional changes that occur from the time of fertilization till the seeds become ready for harvest. Close association between fruit maturation and seed maturation has been reported in leguminous crops like cowpea [3] and black gram [4]. The pod colour in *Crotalaria* changed from light green in early stages to brown around 40-45 DAA and to dull black around 50-60 days and this colour change could be attributed to maturation of pods at different stages of development (Figs. 1&2). Pod length in all the four genotypes increased up to H<sub>3</sub> and H<sub>5</sub> stage with a slight decrease at final stages of maturity. The decrease in length may be due to shrinkage of tissues with the onset of senescence. A similar trend was reported in another green manuring crop *Sesbania* [5].

Seed colour also serves as an effective visual morphological index for physiological maturity. The developing seeds were light green and shiny up to 30 DAA and changed to yellowish/blackish green around 35 DAA. Between 40 and 50 DAA, the seed colour changed to gray or yellow, depending upon the characteristics of the variety as Crota H-1 and L-1 have gray seeds on maturity and Crota ND-1 and Pant-1 have yellow seeds (Figs. 1&2).

The mean number of seeds per pod was highest (9.3) in pods harvested at 20-25 DAA i.e. early stage of development while their number reduced (6.0) in pods harvested at maturity in the early type in both years. In the late type the maximum number was 11.15 at 30 DAA in the year 2003 and 8.8 at 45 DAA in the previous year (Tables 1 and 2). However, at maturity this difference was narrowed down to an average of 7 seeds per pod in all genotypes. The reduction in number of seeds may be due to failure in development of few of the seeds or early abortion of embryo. Similar observations have been reported in *Sesbania* also [5]. A perusal of the data for both the years showed that irrespective of the cultivars and the climatic factors about 30-35 per cent of the fertilized ovules failed to develop into a mature seed in the above genotypes.

Test weight (100 seed weight) of seed is considered as an important parameter that determines the quality of a seed lot. The fresh weight of seed was maximum at 30-35 DAA and then it gradually decreased. The water content may be a factor for higher seed weight during initial developmental stages. In early varieties, the gain in seed weight was found faster i.e. from 5.75g (20 DAA) to 10.40g (30 DAA) in 2002 and almost similar trend was observed in 2003 also. In the late maturing varieties, Crota ND-1 and Pant-1 the gain in seed weight (maximum 10.84g and 12.92g at 35 and 55 DAA in 2002 and 2003 respectively) as well as its loss was more gradual than that in the early varieties (Fig. 3a and Tables 1, 2). This could be correlated with the weather conditions (Fig. 5) especially temperature and relative humidity at seed maturation period. The moderate weather conditions prevailed in the year 2003 extended the duration of seed maturation by the gradual loss of water content in the late maturing types, which in turn might have helped in higher dry matter accumulation and more test weight.

The fresh seeds attained germinability at the 25 DAA onwards in early type and at 30-35 DAA in late type in 2002 (Table 1). The germination percentage at that time was minimum (10.75%) due to more number of immature or under developed seeds, lesser dry matter accumulation and also they

were more prone to surface infection. The increase in days to collection from DAA was positively associated with decrease in number of abnormal seedling or dead seeds. Genotypic difference on germinability and dry matter accumulation was also clearly evident in the data (Table 1). For example, Crota H-1 and L-1 had maximum germination at 35-40 DAA (87.5%), but Crota ND-1 and Pant-1 reached peak germination (>90%) at 45-50 DAA. The developing seeds could germinate at 25-30 DAA in early types in the year 2003 whereas in late varieties, it was delayed to 40-45 DAA suggesting that the stage of development of seed is dependent on various other factors also (Table 2). For early varieties, maximum germination was attained at 40 and 55 DAA in the year 2002 and 2003 respectively and in late types, maximum germination was observed at 50 and 70 DAA. Similar observations [6] were made in *Crotalaria pallida* Ait where seed germinability gradually reached upto a maximum from 25-70 DAF (days after flowering) but decreased thereafter support the present study. Austin [7] and Delouche [8] have emphasized that full germination and viability of seed cannot be attained until the seed

reaches full maturity. In the four genotypes studied maximum germination was observed when the seed moisture content was between 50-60 per cent in both the years. As the water content of seed started decreasing below this critical value the germination per cent started decreasing due to the onset of hard seed development (Fig. 3b, 3c and 3d).

The onset of seed coat imposed dormancy is a later event in the maturation process as has been reported in earlier studies also [1]. The seeds of *Crotalaria* could germinate at 25 DAA and reached maximum at >40DAA, further it was retained until natural drying commences when some of the seeds become dormant. The loss in moisture content seems to be a factor responsible for this initiation of coat imposed dormancy. Mehrotra *et al.* [9] reported genotypic variation in seed dormancy in sunflower. Genotypic difference was recorded in the development of hard seeds in *Crotalaria* also (Table 1). In Crota H-1 and L-1 it started at 40-45 DAA while in Crota ND-1 and Pant-1 at 50-55 DAA. Maximum hard seeds were observed in Crota H-1 and L-1 at 45 DAA when seed moisture content was 9.37 per cent, while in Crota ND-1 and Pant-1, maximum hard seeds were observed

Table 1. Pod and seed parameters in relation stages of maturity in *Crotalaria* in kharif 2002

| DAA    | Pod length (cm) |      | No. seed/pod |      | 100 seed wt (g) |       | Moisture (%) |       | Germination (%) |       | Hard seed (%) |       | Seedling length (cm) |       | Dry wt. (mg) |        |
|--------|-----------------|------|--------------|------|-----------------|-------|--------------|-------|-----------------|-------|---------------|-------|----------------------|-------|--------------|--------|
|        | Early           | Late | Early        | Late | Early           | Late  | Early        | Late  | Early           | Late  | Early         | Late  | Early                | Late  | Early        | Late   |
| H1-20  | 2.75            | 2.83 | 8.10         | 7.40 | 5.75            | 4.67  | 80.91        | 82.36 | 0.00            | 0.00  | 0.00          | 0.00  | -                    | -     | -            | -      |
| H2-25  | 2.87            | 2.86 | 9.30         | 7.85 | 9.04            | 8.54  | 76.91        | 80.60 | 10.75           | 0.00  | 0.00          | 0.00  | -                    | -     | -            | -      |
| H3-30  | 2.99            | 2.87 | 9.05         | 8.05 | 10.40           | 9.47  | 71.52        | 78.45 | 48.75           | 6.00  | 0.00          | 0.00  | 7.65                 | -     | 55.00        | -      |
| H4-35  | 2.88            | 2.89 | 8.70         | 8.05 | 8.40            | 10.84 | 61.29        | 74.97 | 87.00           | 22.50 | 0.00          | 0.00  | 18.28                | -     | 130.00       | -      |
| H5-40  | 2.76            | 3.11 | 8.20         | 8.15 | 4.23            | 10.02 | 20.91        | 66.31 | 87.50           | 66.75 | 7.00          | 0.00  | 27.15                | 15.88 | 150.00       | 115.00 |
| H6-45  | 2.74            | 3.02 | 7.85         | 8.80 | 3.64            | 8.55  | 9.37         | 68.07 | 59.75           | 90.75 | 38.25         | 0.00  | 27.34                | 21.91 | 150.00       | 140.00 |
| H7-50  | 2.71            | 2.93 | 6.50         | 8.65 | 3.62            | 7.52  | 9.27         | 50.99 | 67.00           | 96.25 | 29.75         | 0.50  | 23.91                | 24.25 | 150.00       | 170.00 |
| H8-55  | 2.70            | 2.82 | 6.35         | 7.60 | 3.63            | 5.73  | 9.00         | 43.11 | 69.25           | 86.00 | 26.25         | 12.25 | 21.00                | 23.28 | 135.00       | 170.00 |
| H9-60  | 2.69            | 2.71 | 6.25         | 7.65 | 3.66            | 4.43  | 8.82         | 34.73 | 72.00           | 73.50 | 24.25         | 25.50 | 17.44                | 22.08 | 125.00       | 170.00 |
| H10-65 | 2.68            | 2.70 | 6.05         | 7.25 | 3.21            | 4.26  | 6.25         | 18.82 | 71.50           | 71.75 | 25.00         | 27.00 | 16.36                | 21.17 | 120.00       | 150.00 |
| H11-70 | 2.65            | 2.65 | 6.00         | 7.00 | 3.17            | 4.07  | 6.02         | 9.79  | 70.75           | 69.00 | 26.25         | 29.25 | 15.99                | 19.37 | 115.00       | 135.00 |

Early varieties: Crota H-1 and Crota L-1; Late varieties: Crota ND-1 and Crota Pant-1; DAA: Days after anthesis

Table 2. Pod and seed parameters in relation to stages of maturity in *Crotalaria* in kharif 2003

| DAA    | Pod length (cm) |      | No. seed/pod |       | 100 seed wt. (g) |       | Moisture (%) |       | Germination (%) |       | Hard seed (%) |      | Seedling length (cm) |       | Dry wt. (mg) |        |
|--------|-----------------|------|--------------|-------|------------------|-------|--------------|-------|-----------------|-------|---------------|------|----------------------|-------|--------------|--------|
|        | Early           | Late | Early        | Late  | Early            | Late  | Early        | Late  | Early           | Late  | Early         | Late | Early                | Late  | Early        | Late   |
| H1-20  | 2.80            | 3.25 | 9.20         | 10.80 | 4.85             | 2.99  | 80.72        | 82.76 | 0.00            | 0.00  | 0.00          | 0.00 | -                    | -     | -            | -      |
| H2-25  | 2.90            | 3.35 | 8.60         | 11.05 | 7.72             | 4.63  | 80.95        | 82.33 | 1.00            | 0.00  | 0.00          | 0.00 | -                    | -     | -            | -      |
| H3-30  | 3.00            | 3.25 | 8.15         | 11.15 | 9.75             | 7.72  | 76.92        | 81.42 | 3.25            | 0.00  | 0.00          | 0.00 | -                    | -     | -            | -      |
| H4-35  | 2.90            | 3.20 | 7.65         | 10.25 | 10.96            | 7.98  | 72.3         | 80.75 | 24.00           | 0.00  | 0.00          | 0.00 | -                    | -     | -            | -      |
| H5-40  | 3.00            | 3.20 | 7.60         | 10.20 | 10.02            | 11.02 | 69.81        | 78.22 | 29.00           | 2.37  | 0.00          | 0.00 | -                    | -     | -            | -      |
| H6-45  | 2.90            | 3.10 | 7.25         | 9.90  | 8.04             | 12.28 | 63.24        | 76.65 | 61.75           | 10.25 | 0.00          | 0.00 | 13.50                | -     | 120.00       | -      |
| H7-50  | 2.80            | 3.05 | 6.80         | 9.70  | 6.51             | 12.90 | 51.59        | 69.88 | 74.00           | 21.25 | 0.50          | 0.00 | 16.39                | -     | 133.75       | -      |
| H8-55  | 2.70            | 3.00 | 6.70         | 9.35  | 4.86             | 12.92 | 36.33        | 67.40 | 91.50           | 76.75 | 4.12          | 0.00 | 21.12                | 13.39 | 172.50       | 147.50 |
| H9-60  | 2.65            | 2.95 | 6.50         | 8.45  | 4.36             | 11.83 | 17.60        | 64.30 | 90.25           | 78.5  | 5.25          | 0.00 | 22.00                | 18.82 | 175.00       | 155.00 |
| H10-65 | 2.55            | 2.85 | 6.25         | 7.80  | 4.16             | 11.11 | 9.57         | 57.29 | 88.00           | 80.00 | 7.00          | 0.00 | 22.75                | 22.18 | 180.00       | 160.25 |
| H11-70 | 2.50            | 2.75 | 5.75         | 7.25  | 4.00             | 8.54  | 6.96         | 24.00 | 87.50           | 82.25 | 10.00         | 0.00 | 23.28                | 23.66 | 182.50       | 172.50 |

Early varieties: Crota H-1 and Grata L-1; Late varieties: Crota ND-1 and Crota Pant-1; DAA= Days after anthesis

at 70 DAA when seed moisture content was 9.79 per cent (Fig. 3d). Development of hard seeds started at 50-55 DAA in early types whereas no dormancy was observed in late types till 70 DAA. It is evident from the two years data that development of hard seeds depends upon genotype as well as environmental factors during desiccation. It was observed that degree of maturity in seed influenced the duration of dormancy and usually seed become impermeable during later stages of maturation [10, 11].

Seeds in the early stages of development had higher moisture content and with the gradual progress towards the maturity the seed moisture content decreased from 80.91 per cent at 20 DAA to 6.02 per cent at 70 DAA in early types and 82.36 per cent (20 DAA) to 9.79 per cent (70 DAA) in late types in the year 2002. While in 2003 the initial values were similar but at 70 DAA the values were 6.96 per cent and 24.0 per cent respectively for early and late types (Fig. 3b). These results are comparable with that in *Sesbania* [4] and in pigeon pea [12].

The differences among the genotypes were also

evident in the rate of dehydration of maturing seeds. The reduction in water content of seed was gradual up to 35 DAA and then onwards it showed a sudden drop in the early varieties leading to physiological maturity. While in late types the drop in moisture was gradual throughout and was evident only after 50 DAA, which can be attributed for the delay in maturity as well (Fig. 3b). The rapid dehydration can be considered as maturation drying process affected by external factors. According to Bewley and Black [13] maturation drying is an integral part of development in most of the seeds and in fact development is considered to be complete when the seed has dried.

In the year 2002 the sudden moisture loss was associated with development of hard seeds also and both the events i.e. sudden loss in seed moisture and hard seed development coincided very well. In the year 2002 during the seed maturation period the maximum (40°C) and minimum (25°C) temperature was much higher than that in the following year (Fig. 4). The year 2002 was also considered as a drought year. While in year 2003 the maximum temperature was around 30°C and minimum was much below 20°C



Fig. 1. Pod and seed at different maturity stages in early type

Fig. 2. Pod and seed at different maturity stages in late type

(Fig. 5) during seed maturation period, it was on an average  $10^{\circ}\text{C}$  below than the previous year. This higher temperature and drought like situation in the year 2002 seems to have contributed to sudden loss in seed moisture and to the development of hard seeds in both early and late types. While in 2003 the mild climatic conditions throughout crop season might have contributed to the reduced percentage of hard seeds in the early varieties and complete absence of it in the late types suggesting the role of environmental conditions on hard seed development in *Crotalaria*.

High temperatures, low air relative humidity [14] and water stress [15] favour hard seed production in different species. The proportion of hard seeds produced by legume species varies with year and geographic region [16] also in many species the proportion of hard seeds increased as the seed size decreased [17, 18].

The mean seedling length and dry weight, the two indices of seed vigour, reached maximum at 45 DAA for early types, whereas in late types, the values for seedling length reached maximum at 50

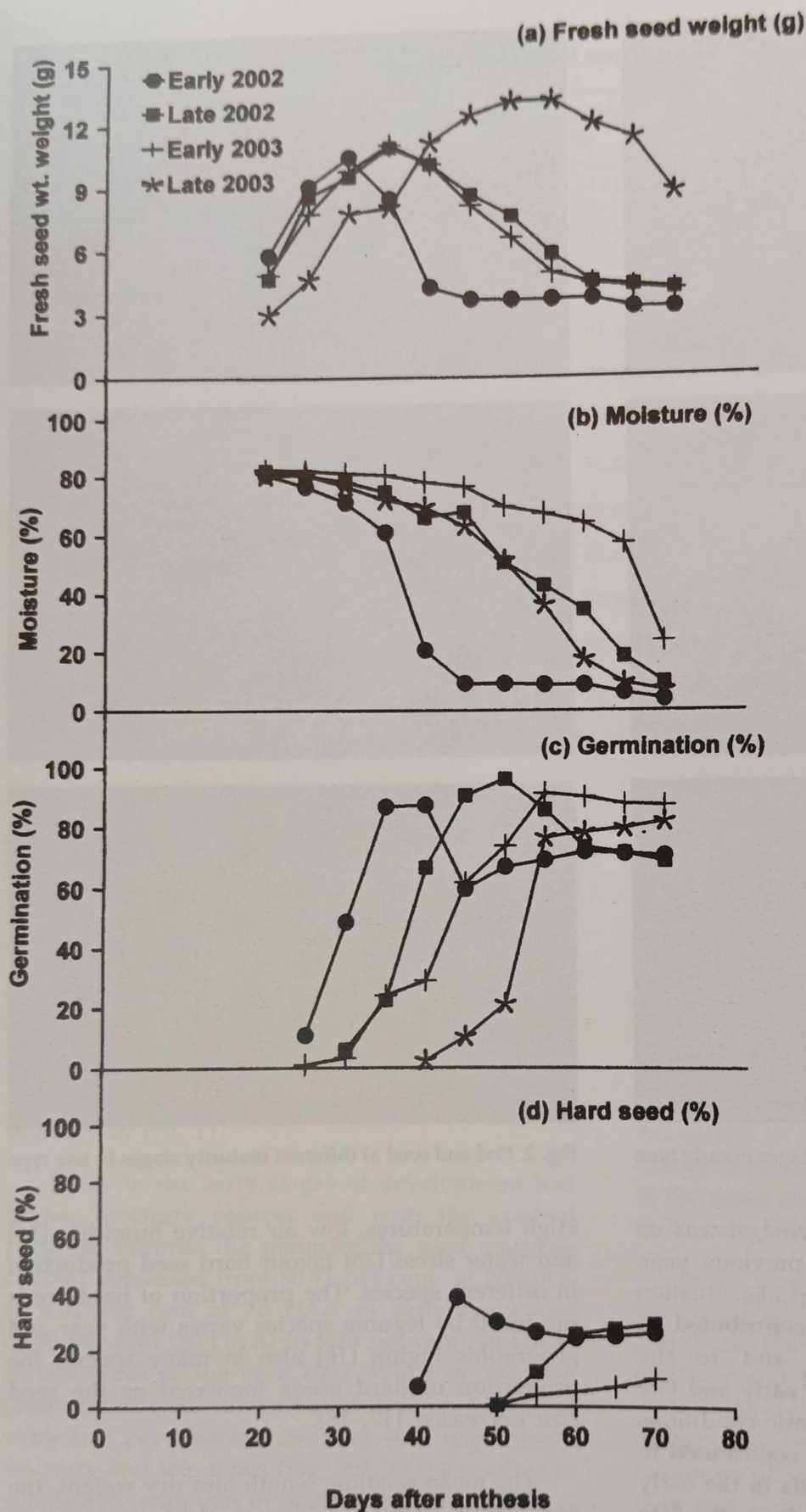


Fig. 3. Seed quality parameters (a) Fresh seed weight, (b) Moisture, (c) Germination and (d) Hard seed in relation to maturity in Crota

DAA and decreased towards maturity in 2002 (Table 1). In 2003, maximum seedling length and dry weight were observed at 70 DAA for early as well as late varieties (Table 2). These higher values coincided very well with the physiological maturity. Dharmalingam and Basu [19] also reported highest germination and vigor of seeds harvested at physiological maturity than those harvested prematurely.

Seeds of Crota H-1 and L-1 attained harvestable maturity at 50 DAA whereas in Crota ND-1 and Pant-1 it took 70 DAA for it and almost same time difference was there between the two groups for the start of flowering too. So the total duration from sowing to harvesting was approximately 100 and 150 days respectively for the early and late types. The genotypic variation among the cultivars for the initiation of flowering, seed development and maturity can ultimately affect the seed quality especially where extreme climatic conditions prevails.

Among the four genotypes studied, the seeds reached maximum vigour and viability between 40<sup>th</sup> and 50<sup>th</sup> DAA; seed moisture content still being high at these stages. The field maturity, therefore, is attained around 50 and 70 DAA respectively for early

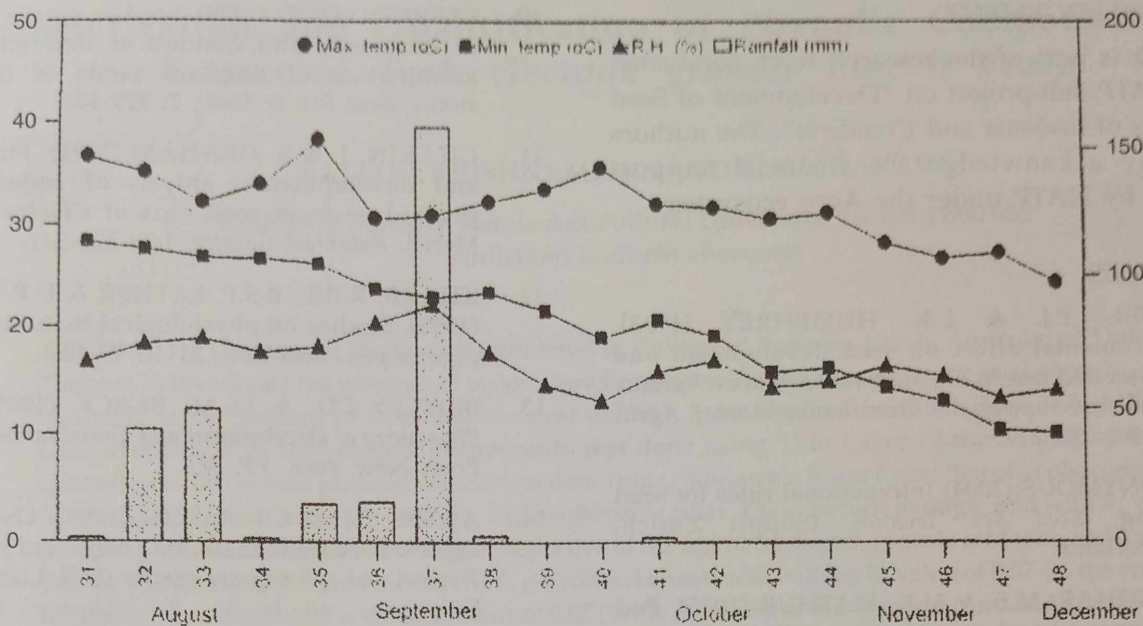


Fig. 4. Mean weekly meteorological variables during *kharif* 2002

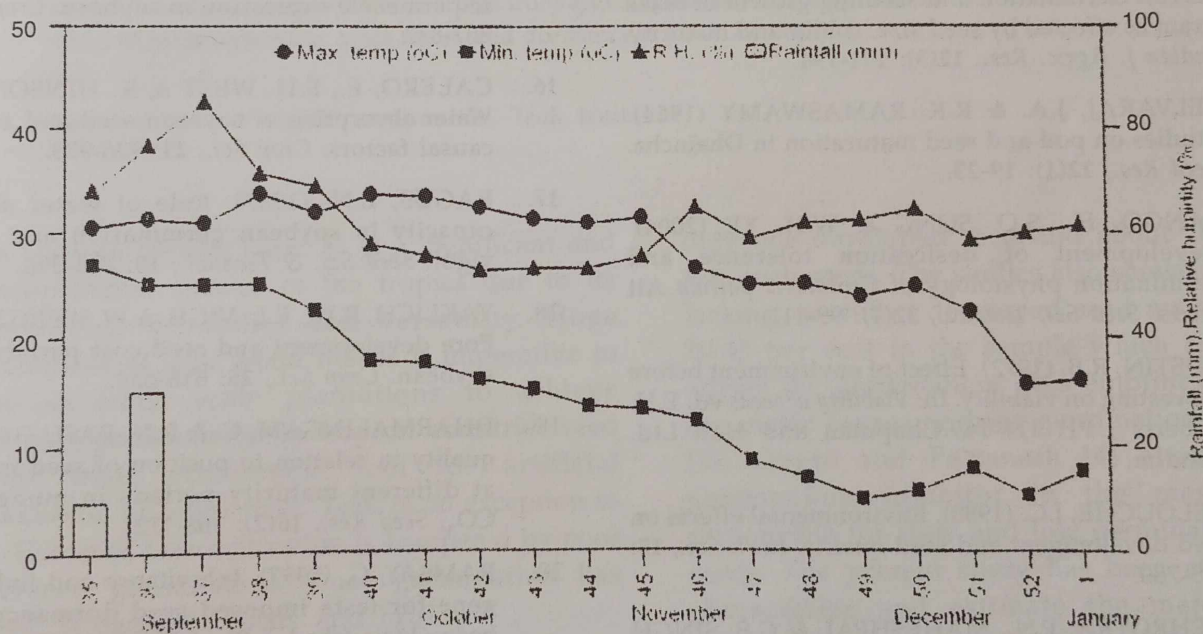


Fig. 5. Mean weekly meteorological variables during *kharif* 2003

and late type, which is the proper time for the harvesting of the crop. The same was also indicated by physical changes in the pod and seed coat color. The overall seed quality was not affected by the prevailing environmental factors during seed maturation stage as evidenced from various seed quality parameters. However, the development of physical seed dormancy (hard seeds) is very much

influenced by the rate of moisture loss (desiccation) in the maturing seeds, which is influenced by the prevailing weather conditions. Seed coat hardness though a heritable character is highly influenced by environmental factors as reported earlier [20] supports the present study in *Crotalaria*.

## ACKNOWLEDGEMENT

The work is part of the research work conducted in the NATP Sub-project on "Development of Seed Standards of *Sesbania* and *Crotalaria*". The authors thankfully acknowledge the financial support provided by NATP under the Agro ecosystem.

## REFERENCES

1. ARGEL, P.J. & L.R. HUMPHREY (1983). Environmental effect on seed development and hard seededness in *Stylosanthes humata* cv. Verano. II Moisture supply and illuminance. *Aust. J. Agric. Res.*, **34**: 271-277.
2. ANONYMOUS (2004) International rules for seed testing. *Seed Sci. Technol.* (Suppl) Zurich, Switzerland.
3. MANOHAR, M.S. & M.K. MATHUR (1975). Pod development and germination studies on cowpea (*Vigna sinensis* Savi.). *Seed Res.*, **3**(1): 29-33.
4. RAO, S.P., A.APPA RAO & R.K. MUKHERJEE (1978). Germination and seedling growth of black gram as affected by seed size, colour and maturity. *Indian J. Agric. Res.*, **12**(3): 191-194.
5. SELVARAJ, J.A. & R.K. RAMASWAMY (1984). Studies on pod and seed maturation in Dhaincha. *Seed Res.*, **12**(1): 19-23.
6. YANGQ, H., S.Q. SONG & W.H. YE (2004). Development of desiccation tolerance and germination physiology of *Crotalaria pallida* Ait seeds. *Seed Sci. Technol.*, **32**(1): 99-111.
7. AUSTIN, R.B. (1972). Effect of environment before harvesting on viability. In: *Viability of seeds* ed. E.H. Roberts , PP. 114-149. Chapman and Hall Ltd. London.
8. DELOUCHE, J.C. (1980). Environmental effects on seed development and seed quality. *Hort. Sci.*, **15**: 775-780.
9. MEHROTRA, P.N., MAHESHPAL & G.S. SINGH (1978). Studies on post harvest dormancy in sunflower seeds. *Seed Res.*, **6**: 91-93.
10. CSERESNYES Z. (1979). Studies on the duration of dormancy and method of determining the germination of dormant seeds of *Helianthus annuus*. *Seed Sci. & Tech.*, **7**: 179-188.
11. GILLKIN, J. & S. GRAHAM (1991). Purification and developmental analysis of major anionic peroxidase from seed coat of *Glycine max* (L.) Merril. *Botanical Gazette*, **148**: 324-331.
12. KHARB, R.P.S., B.S.P. LATHER & D.P. DESWAL (1993). Studies on physiological maturity in early pigeon pea. *Seed Res.*, **21**(1): 45-48.
13. BEWLEY J.D. & J.S.M. BLACK (1985). *Seeds-Physiology of Development and Germination*, Plenum Press New York. PP. 347.
14. ARGEL, P.J. & C.J. PATON (1999). Overcoming legume hardseededness. In: *Forage seed production: Tropical and sub-tropical species* (D.S. Loch and J.E. Ferguson, eds.). V. 2, chapter 14, CAB International, Wallingford, p. 247-265.
15. HILL, H.J., S.H. WEST & K. HINSON (1986). Effect of water stress during seed fill on impermeable expression in soybean. *Crop Sci.*, **26**: 807-812.
16. CALERO, E., S.H. WEST & K. HINSON (1981). Water absorption of soybean seed and associated causal factors. *Crop Sci.*, **21**: 926-933.
17. RAGUS, L.N. (1987). Role of water absorbing capacity in soybean germination and seedling vigor. *Seed Sci. & Technol.*, **15**: 285-296.
18. YAKLICH, R.W., E.L. VIGIL & W. WERGIN (1986). Pore development and seed coat permeability in soybean. *Crop Sci.*, **26**: 616-624.
19. DHARMALINGAM, C. & R.N. BASU (1988). Seed quality in relation to position of seed in the pod at different maturity periods in mungbean cv. CO<sub>3</sub>. *Seed Res.*, **16**(2): 168-172.
20. RAMSAY, G. (1997). Inheritance and linkage of a gene for testa imposed seed dormancy in faba bean (*Vicia faba* L.) *Plant Breeding*, **116**: 287-289.