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RESEARCH ARTICLE

Phenophasic variation in cotyledon chlorophyll content and chlorophyll fluorescence: An insight into germinating cowpea

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Abstract

Cowpea is an important grain and vegetable legume that is grown under water deficit-prone tropical and subtropical regions. These regions require genotypes of cowpea that can be established rapidly at the seedling stage. A better understanding of the phenomenon that happens during cowpea germination can help us to develop the varieties that have traits to establish early in the soil. The present study was conducted to investigate the phenophasic variation in cotyledon chlorophyll content and chlorophyll fluorescence in the germinating cowpea at different developmental stages because both are indicators of photosynthetic capacity and compared to gas exchange parameters; they are high throughput, speedy and simple in nature. Total chlorophyll content and chlorophyll fluorescence parameters *viz.* maximum fluorescence (F_m), the maximum quantum efficiency of PSII (F_v/F_m), potential photosynthetic activity of PSII (F_v/F_0), steady-state relative fluorescence decline ratio (R_{fd}_Lss) and steady-state coefficient of photochemical quenching based on lake model (qL_Lss) were measured in the cotyledons of germinating cowpea seedlings at the three phenophasic stages that are named as closed, opened and unfurled primary leaf (UPL) cotyledon stages. It was revealed that total chlorophyll content, F_m, F_v/F_m, F_v/F_0, R_{fd}-Lss and qL_Lss are significantly higher in UPL cotyledons as compared to closed cotyledons across all the cowpea genotypes which are the reliable indicators of photosynthetic efficiency. Moreover, total chlorophyll content in cowpea cotyledon was also found to be significantly and positively correlated with chlorophyll fluorescence parameters. The present study suggeststhat UPL cotyledons have better-developed photosynthesis apparatus, and plants tend to be independent of cotyledonary storage for their growth at this phenological stage. Apart from that, it was also evident that there is a natural variation of chlorophyll fluorescence parameters in the cowpea cotyledons with a genetic basis that can be utilized in breeding and selection programs of early establishing cowpea genotypes where chlorophyll fluorescence can be used as an selection tool.

Keywords: Cowpea, Cotyledon, Chlorophyll fluorescence, F_v/F_m, R_{fd}.

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Introduction

Plant passes through various phenophases to complete their life cycle, and among them, seed germination and seedling development are important phenolphases that play a pivotal role in plant establishment, survival and dispersal (Carrera-Castano et al., 2020). Cotyledons are the important components of seeds that play an important role in seed germination and seedling development (Shi et al., 2020). Dicotyledonous plants show the epigeal mode of germination and during the germination process, cotyledons appear as the first pair of leaves, which serve as both storage-mobilizing and photosynthetic organs (Cao et al., 2022). During the epigeal mode of germination, at the initial stage, when the seedling remains inside the soil, proplastids are converted into etioplasts, but once seedlings come out of the soil and gain contact with light, etioplasts transformed into chloroplasts, which enable the cotyledon to photosynthesize (Albrecht et al., 2006). Altogether, at the initial phase of seedling development, the major portion of assimilates is provided by the cotyledons until the first true leaf becomes a significant exporter of photosynthates

(Xu et al., 2021). The importance of the photosynthetic activity of cotyledons at the early seedling growth has been emphasized by several authors, particularly the plants having seeds with epigeal germination (Zheng et al., 2011; Wang et al., 2019). Cowpea [*Vigna unguiculata* (L.) Walp] is an important annual legume crop that shows the epigeal mode of germination. It originated in Africa and is widely cultivated in tropical and subtropical regions of the world (Herniter et al., 2020; Omomowo and Babalola, 2021). In India, ICAR-IIVR, Varanasi, UP dominates in the development, release and commercialization of varieties of bush-type vegetable cowpeas (Lal et al., 2017; 2018). During germination, the photosynthetic behavior at different phenophasic stages is considered vital for establishing cowpea seedlings. Moreover, the photosynthetic characteristic of plants is an effective way to dissect the physiological conditions of plants.

Chlorophyll fluorescence (ChlF) imaging is a non-destructive and rapid tool that allows the non-contact measurements of photosynthesis (Stirbet and Govindjee, 2011, 2012). Previous studies have revealed that chlorophyll fluorescence parameters, such as initial fluorescence (Fo), maximal fluorescence (F_m), variable fluorescence (F_v, i.e. F_m-F_0), maximum quantum efficiency of PSII (F_v/F_m), potential photosynthetic activity of PSII (F_v/F_0), steady-state relative fluorescence decline ratio (R_{fd}_Lss) and steady-state coefficient of photochemical quenching based on lake model (qL_Lss) are the reliable indicators to evaluate the photosynthesis metabolism in the plants (Rao et al., 2021; Legendre et al., 2021). In this direction, the present study was conducted to quantify the chlorophyll fluorescence characteristics of cowpea cotyledons at different phenophasic stages to reveal their photosynthetic responses during germination.

Materials and Methods

Plant materials and growth conditions

The present study was conducted at ICAR-National Institute of Abiotic Stress Management, Baramati, India (coordinates of place are 18.1592° N, 74.5007° E). Seeds of 6 different cowpea genotypes/varieties (Kashi Nidhi, Jawahar Cowpea 1, Arka Garima, VRCP-12, VRCP-49-5 and Kashi Kanchan) were sown in a small glass pot containing single seed and soilrite (containing a mixture of perlite, peat and exfoliated vermiculite in equal ratio) as growth medium and followed by growth in plant growth chamber. The temperature, relative humidity, CO_2 concentration and photosynthetically active radiation (PAR) of the plant growth chamber maintained during the experiment are given in Table 1. The pots were maintained at field capacity after regular intervals. Cowpea seeds were germinated after two days of sowing, and these germinated seeds were further examined. This experiment was repeated four times before writing the manuscript.

Table 1: Conditions of Plant Growth Chamber

Parameters	*Day*	*Night*
Duration (hrs)	14	10
Temperature (°C)	27	20
CO_2 Conc. (ppm)	300	300
Light internsity (μ mol)	700	0

Total chlorophyll content and chlorophyll fluorescence measurements

Total chlorophyll content and chlorophyll fluorescence parameters were measured in the cotyledons of germinating cowpea seedlings at the three phenophasic stages that are named as closed, opened and unfurled primary leaf (UPL) cotyledon (Here UPL cotyledon stage depicts just after the unfurling of the primary leaf) (Figure 1). Total chlorophyll content (μg g^{-1} FW) was determined by using the method described by Hiscox and Israelstam (1979). Handy FluorCam FC 1000-H, PSI, Brno, Czech Republic obtained chlorophyll fluorescence parameters of cotyledons. The parameters are set to shutter, 1; sensitivity, 28%; super, 90%; far, 50%; act, 1, 100%. The fluorescence parameters measured are listed in Table 2. The same seedling was used for chlorophyll fluorescence measurement at different growth stages *viz.* closed, opened and UPL cotyledon stages. Ten replicate measurements were taken for each treatment. The samples were dark-adapted for 30 min in a dark chamber before chlorophyll fluorescence measurements. The chlorophyll fluorescence measurements were determined by the method described by Rao et al. (2021).

Statistical analysis

The results are expressed as means with standard error (S.E.). The significance difference (at p = 0.05) across the genotypes for closed, opened and UPL cotyledon stages was determined by Duncan's multiple-range tests for total chlorophyll content and chlorophyll fluorescence parameters. ANOVA and critical difference values were calculated by using SPSS 27.

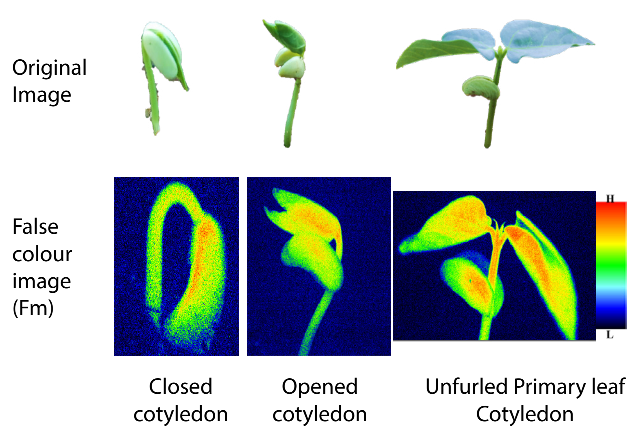

Figure 1: Phenophase stages of cowpea seedling after germination and their representative fluorcam false colour images

Table 2: Detailed information on the chlorophyll fluorescence indexes

Symbol	*Formula*	*Name*	*Description*
F_m	Measured	Maximum fluorescence in a dark-adapted state	QA reduced (qP = 0), non-photochemical quenching relaxed (NPQ = 0)
F_v/F_m	F_m-F_o/F_m	Maximum quantum yield of PSII	Maximum PSII quantum yield in a dark-adapted state
F_v/F_o	F_m-F_o/F_0	Potential photosynthetic activity of PSII	The change in PSII-absorbed light energy to reduce the efficiency of QA and potential PSII activation
R_{fd}_Lss	(F_p – F_t_Lss)/F_t_Lss	Steady-state relative fluorescence decline ratio	Empiric parameter used to assess plant vitality
qL_Lss	[(F_m_Lss – F_t_Lss)/ (F_m_Lss – F_0_Lss)]* F_0_Lss/ F_t_Lss	Stead-state coefficient of photochemical quenching based on Lake Model	Estimate the fraction of open PSII centres.

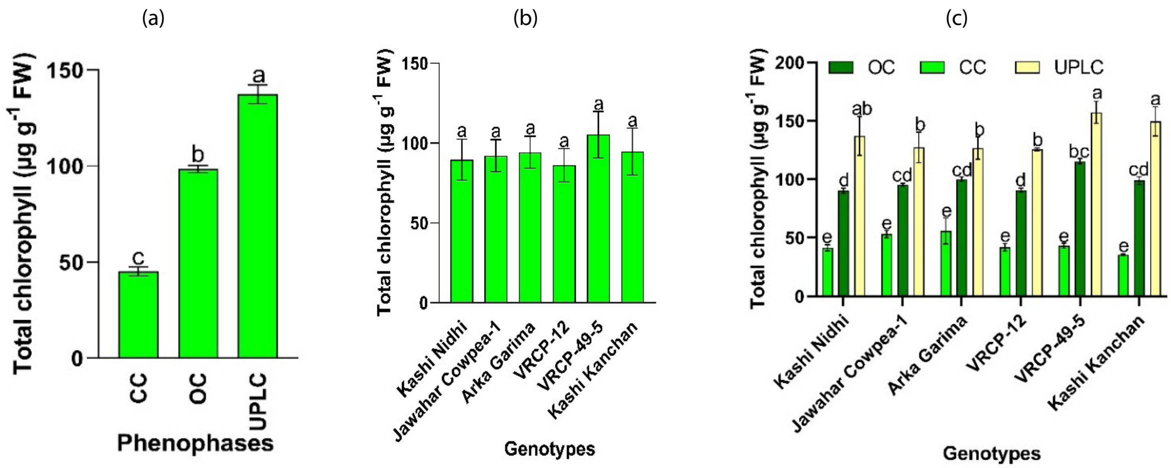

Figure 2: Phenophasic (a), genotypic (b) and phenophase X genotype interaction effect (c) on total chlorophyll content (µg g^{-1} FW) in cowpea at early growth stages. CC: Closed cotyledon, OP: Opened cotyledon and UPLC: Unfurled primary leaf cotyledon.

Results and Discussion

During the light reaction of photosynthesis, photosynthetically active radiations (PAR) are absorbed by pigment-protein complexes, which further transfer their electronic excitation energy towards the reaction centers (PSI and PSII), where primary electric charge separation takes place (Caffarri et al., 2014; Lu, 2016). Therefore, chlorophyll content and chlorophyll fluorescence parameters analysis are considered an important approach to evaluate the health or integrity of the internal apparatus during the photosynthetic process within a leaf (Clark et al., 2000; Mishev et al., 2009), which provides a rapid and accurate technique of detecting the physiological conditions of plants (Jiang et al., 2017; Tsai et al., 2019). Therefore, in the present investigation, we have utilized total chlorophyll content and chlorophyll fluorescence measurements to study the pattern of the development of photosynthesis apparatus in the cowpea cotyledons during the germination process since they play a pivotal role in seedling development.

Phenophasic variation of chlorophyll content in cotyledons

Our results showed that total chlorophyll content (µg g^{-1} FW) is significantly higher in UPL cotyledon as compared to closed and opened cotyledons across all the cowpea genotypes. The chlorophyll content ranges from 35.50 to 55.86 µg g^{-1} FW, 90.27 to 115.36 µg g^{-1} FW and 125.68 to 157.27 µg g^{-1} FW in closed, opened and UPL cotyledons, respectively (Figure 2 a, c). Overall, there was no significant difference in total chlorophyll content among genotypes (Figure 2b). Furthermore, it was noticed that there was no significant difference in chlorophyll content (µg g^{-1} FW) across the genotypes under the closed cotyledon stage, whereas at UPL cotyledon stage VRCP 49-5 and Kashi Kanchan genotypes contain significantly higher total chlorophyll content as compared to other genotypes. Kashi Kanchan and VRCP 49-5 genotypes showed 321.44 and 264.80% increase in total chlorophyll content, respectively at UPL cotyledon with respect to the closed cotyledon stage.

Previous studies have demonstrated that when the seedling comes in contact with light after emergence from the soil, it becomes autotrophic (etioplasts transformed into chloroplast) and triggers the accumulation of chlorophyll, which enables the plant/cotyledon to photosynthesize (Albrecht et al., 2006). We also observed that after the seedling emerges from the soil, there was consequently increased accumulation of chlorophyll content at opened and UPL cotyledon stages (Figure 2a); thus, our study

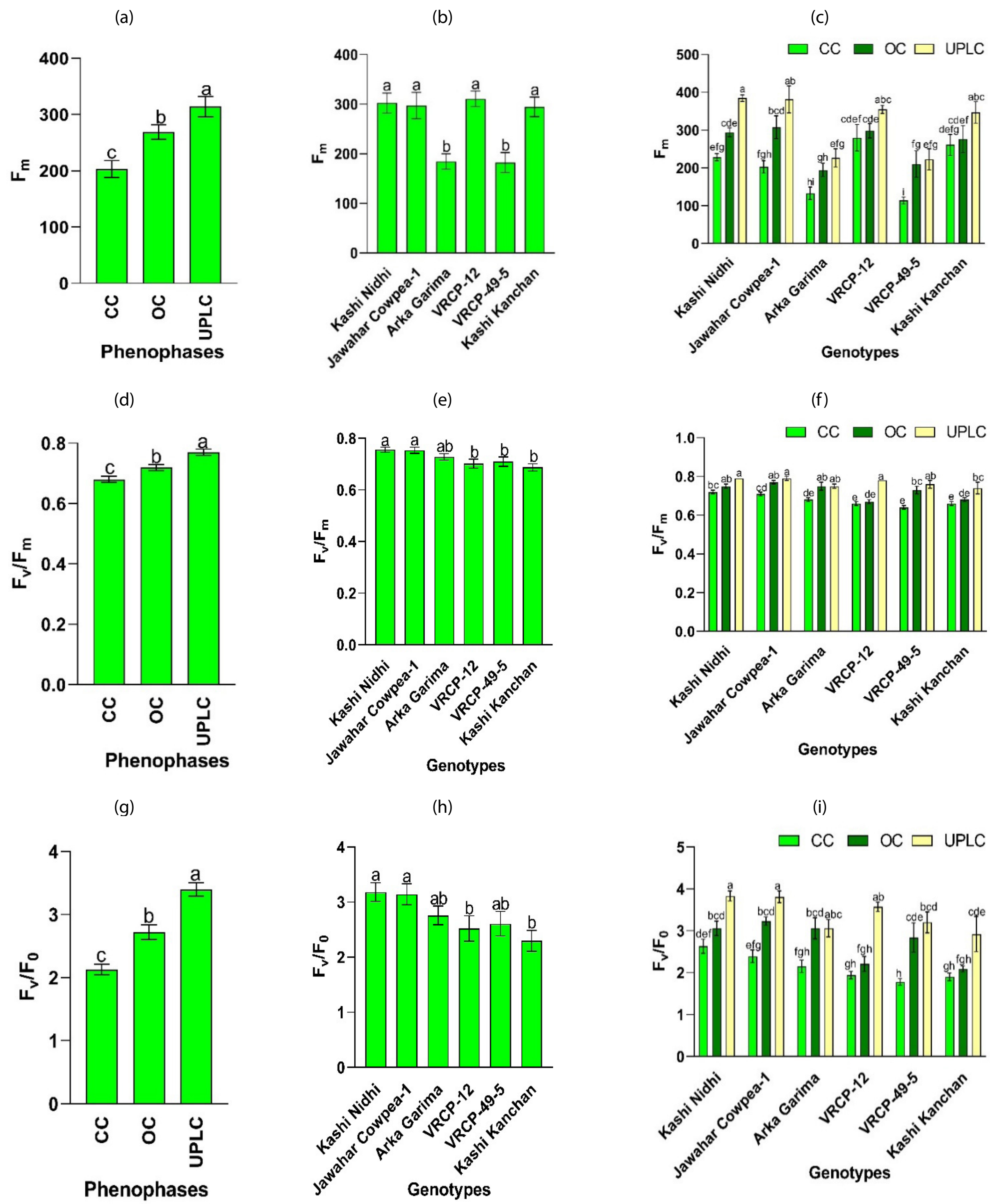

Figure 3: Phenophasic, genotypic and phenophase X genotype interaction effect on F_m (a, b, c), F_v/F_m (d, e, f) and F_v/F_0 (g, h, i) in cowpea at early growth stages. CC: Closed cotyledon, OP: Opened cotyledon and UPLC: Unfurled primary leaf cotyledon.

indicates that biosynthesis of chloroplast pigments is affected by phenophasic stages. The chlorophyll content has a positive relationship with the photosynthetic rate (Zhang et al., 2013) because chlorophyll is involved in light-driven charge separation and transport of electrons during the light reaction of photosynthesis (Rochaix, 2011).

Phenophasic variation of chlorophyll fluorescence kinetics in cotyledons

The health and functioning of the photosynthetic apparatus of cowpea cotyledons at the three respective stages were evaluated by measuring the chlorophyll fluorescence parameters (F_m, F_v/F_m, F_v/F_0, R_{fd}_Lss and qP_Lss). It was

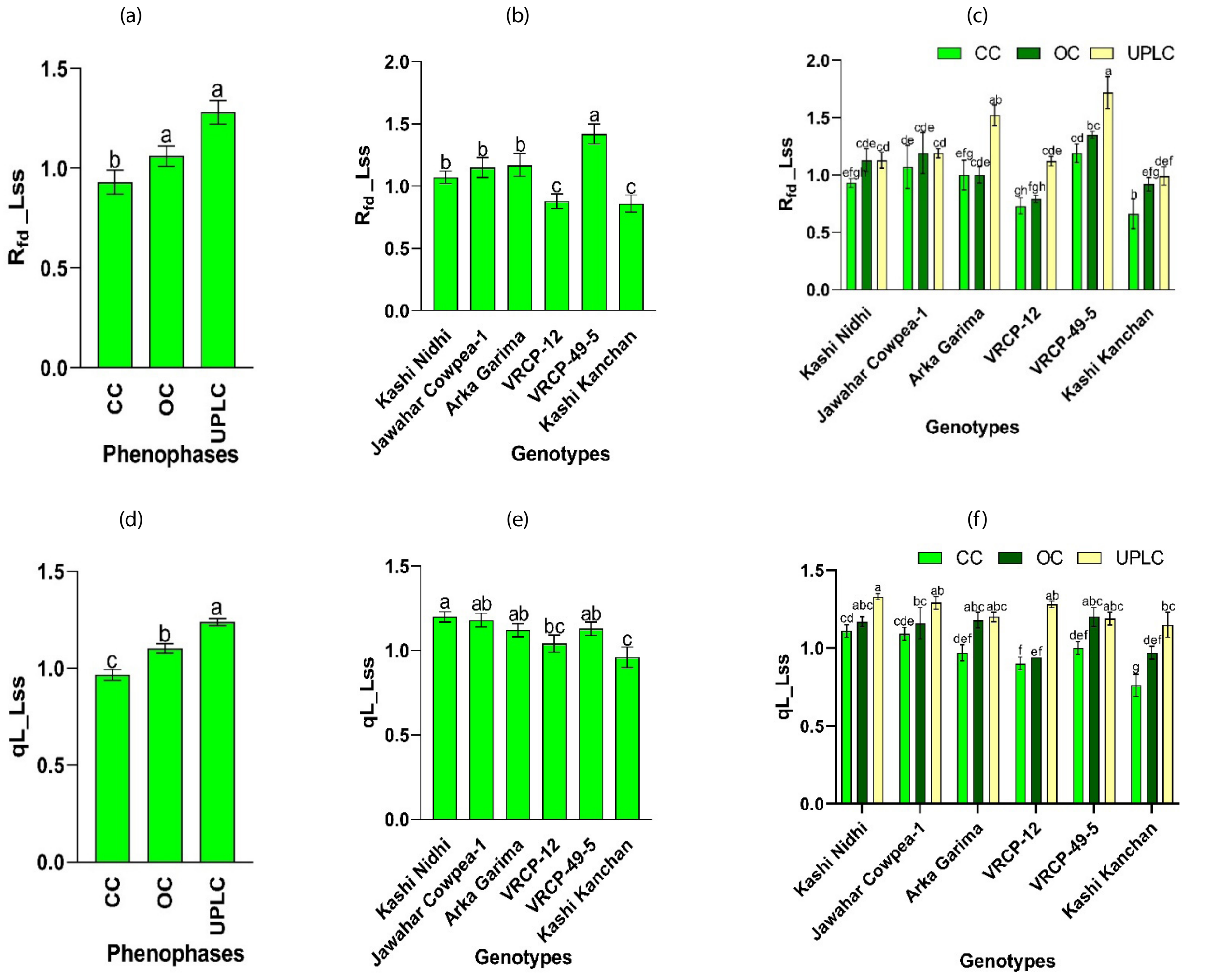

Figure 4: Phenophasic, genotypic and phenophase X genotype interaction effect on R_{fd}_Lss(a, b, c) and qL_Lss (d, e, f) in cowpea at early growth stages.CC: Closed cotyledon, OP: Opened cotyledon and UPLC: Unfurled primary leaf cotyledon.

observed that the F_m, F_v/F_m, and F_v/F_0, values of UPL cotyledons were significantly higher as compared to closed and opened cotyledons across all the cowpea genotypes (Figure 3 a, c d, f, g, i). The highest percent increase of F_m (95.62 %) and F_v/F_m (18.75 %) were found at the UPL cotyledon stage in the VRCP 49-5 genotype. The F_v/F_m values range from 0.64 to 0.72, 0.66 to 0.76, and 0.73 to 0.79 for closed, opened and UPL cotyledons, respectively. Maximum fluorescence (F_m) after dark adaptation reflects the PSII electron transport capacity (Qian et al., 2011). The increased F_m in UPL cotyledon revealed that it has better photosynthetic apparatus compared to closed and opened cotyledons (Friedland et al., 2019). Maximal quantum yield ratio (F_v/F_m) gives information about the photosynthetic efficiency of dark-adapted reaction centers (Hou et al., 2015), and directly reflects the health of the PSII reaction center as well as the potential maximum photosynthetic capacity of plants. The F_v/F_m value ranges from 0.80 to 0.84 in higher plants and very small changes occur under unstressed conditions (Sánchez-Moreiras et al., 2020; Xu et al., 2020). Whereas in this study F_v/F_m values of the UPL cotyledon range from 0.73 to 0.79, which is higher than closed cotyledon. The increased PSII photochemical efficiency (F_v/F_m) in UPL cotyledon may contribute to the improvement of photosynthesis.

Furthermore, it was also revealed that R_{fd}_Lss and qL_Lss are significantly higher in UPL cotyledons as compared to closed and opened cotyledons across all the cowpea genotypes (Figure 4 a-f). Overall, the VRCP-49-5 genotype showed higher R_{fd}_Lss as compared to other genotypes (Figure 4 b). VRCP-49-5 genotypes showed significantly higher R_{fd}_Lss at the UPL cotyledon stage as compared to other genotypes (Figure 4 e, f). Furthermore, R_{fd} and F_v/F_0 are considered more sensitive ratios as compared to F_v/F_m, which gives an accurate idea about the health of photosynthetic apparatus (Lichtenthaler et al., 2005a). As F_v/F_m is measured in a non-functional dark-adapted state, whereas R_{fd} (F_d/F_s) is measured in a functional light-adapted state thus, it is a straightforward indicator of CO_2 fixation and photosynthetic

Table 3: Correlation matrix between total chlorophyll content and chlorophyll fluorescence parameters in cowpea cotyledons

Parameters	F_m	F_v/F_m	F_v/F_0	R_{fd}_Lss	*qL_Lss*	*Total_Chls*
Fm	1	0.528**	0.553**	-0.106	0.314**	0.507**
F_v/F_m		1	985**	0.565**	0.857**	0.685**
F_v/F_0			1	0.562**	0.855**	0.694**
R_{fd}_Lss				1	0.714**	0.501**
qL_Lss					1	0.626**
Total_Chls						1

**Significant at the 0.01 level.

capacity of leaves (Lichtenthaler et al., 2005b; Rousseau et al., 2015). The increased R_{fd} in UPL cotyledons as compared to closed cotyledons across the cowpea genotypes reflects that UPL cotyledons have higher photosynthetic capacity and CO_2 fixation rates as compared to closed cotyledons.

The coefficient of photochemical quenching (qL) quantifies the actual fraction of PSII reaction centers being in the open state (i.e., with reoxidized QA), which is highly correlated with the quantum yield of PSII (Han et al., 2016). Our study also found that qL_Lss is positively correlated with quantum yield (r = 0.874). qL_Lss was significantly higher in UPL cotyledons as compared to closed cotyledons which means that the former has an increased number of open reoxidised QA and thus higher quantum yield. The highest quantum efficiency observed at the UPL cotyledon stage indicates that the plant tends to be independent of cotyledonary storage for its growth at this phonological stage.

Correlation among total chlorophyll content and chlorophyll fluorescence parameters in cotyledons

Pearson's correlation analysis between total chlorophyll content and chlorophyll fluorescence parameters in cowpea cotyledons showed that total chlorophyll content is significantly (p <0.01) and positively correlated with F_m, F_v/F_m, F_v/F_0, R_{fd}_Lss and qL_Lss which are the reliable indicator of photosynthesis rate (Guo and Al-Khatib, 2003) (Table 3). R_{fd}_Lss was negatively correlated with F_m. Amri et al. (2021) have also reported a significant positive correlation between the Chlorophyll content Index (CCI) and F_v/F_m in faba beans.

Genetic variation in phenology influenced chlorophyll fluorescence kinetics in cotyledons

The present study provides a comprehensive investigation of the genetic variation of chlorophyll fluorescence kinetics (F_m, F_v/F_m, F_v/F_0, R_{fd}_Lss and qP_Lss) in the cowpea cotyledons. It was revealed that VRCP-49-5 has the highest R_{fd}_Lss, which is directly caused by the increased total chlorophyll content (Figure 2b and Figure 4b). The significant differences in chlorophyll fluorescence parameters (F_m, F_v/F_m, F_v/F_0, R_{fd}_Lss, and qP_Lss) between these six genotypes suggest that there is natural photosynthetic variation in the cowpea cotyledons with a genetic basis. These genetic differences can be further utilized in breeding and selection programs with chlorophyll fluorescence as a selection tool (Daymond and Hadley, 2004; Balasimha et al., 2013; Prinzenberg et al., 2018).

Conclusion

Chlorophyll fluorescence kinetics observed in this experiment indicate that the phenology of plants greatly influences the PSII activity in cowpea seedlings. The highest quantum efficiency observed at the UPL cotyledon stage indicates that the plant tends to be independent of cotyledonary storage for its growth at this phonological stage. This is further supported by total chlorophyll content in UPL cotyledons, which was higher than the same observed at either the closed cotyledonary stage or the open cotyledonary stage. This was evident in all six genotypes, which did not exhibit genetic variation for this trait in all the technical repeats. Hence, it is suggested that the cowpea, in general, tends to be independent of cotyledonary storage of assimilates only after the first fully unfurled leaf stage as the UPL cotyledons have better-developed photosynthesis apparatus compared to closed cotyledons to support the growth of the seedling. These findings may be useful in precision agriculture aiming at the management of cowpeas at the early stage of establishment. Additionally, the natural variation of chlorophyll fluorescence parameters in the cowpea cotyledons with a genetic basis can be utilized in breeding and selection programs of early establishing cowpea genotypes that are prime requisites for water deficit regions of the world.

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References

Albrecht, V., Ingenfeld, A., & Apel, K. (2006). Characterization of the snowy cotyledon 1 mutant of *Arabidopsis thaliana*: the impact of chloroplast elongation factor G on chloroplast development and plant vitality. Plant molecular biology, 60, 507-518.

Amri, M., Abbes, Z., Trabelsi, I., Ghanem, M.E., Mentag, R., & Kharrat, M. (2021). Chlorophyll content and fluorescence as physiological parameters for monitoring *Orobanche foetida* Poir. infection in faba bean. Plos one, 16(5), p.e0241527.

Balasimha, D., Apshara, S.E., & Jose, C.T. (2013). Genotypic variations in chlorophyll fluorescence and stomatal conductance of cocoa in relation to drought tolerance. Journal of Plantation Crops, 41(1), 40-45.

Caffarri, S., Tibiletti, T., C Jennings, R., & Santabarbara, S. (2014). A comparison between plant photosystem I and photosystem II architecture and functioning. Current Protein and Peptide Science, 15(4), 296-331.

Cao, J., Li, X., Chen, L., He, M., & Lan, H. (2022). The Developmental Delay of Seedlings With Cotyledons Only Confers Stress Tolerance to *Suaedaaralocaspica* (Chenopodiaceae) by Unique Performance on Morphology, Physiology, and Gene Expression. Frontiers in Plant Science, 13, 844430.

Carrera-Castaño, G., Calleja-Cabrera, J., Pernas, M., Gómez, L., & Oñate-Sánchez, L. (2020). An updated overview on the regulation of seed germination. Plants, 9(6), 703.

Clark, A.J., Landolt, W., Bucher, J.B., & Strasser, R.J. (2000). Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. Environmental Pollution, 109(3), 501-507.

Daymond, A.J., & Hadley, P. (2004). The effects of temperature and light integral on early +vegetative growth and chlorophyll fluorescence of four contrasting genotypes of cacao (*Theobroma cacao*). Annals of applied biology, 145(3), 257-262.

Guo, P., & Al-Khatib, K. (2003). Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). Weed Science, 51(6), 869-875.

Herniter, I.A., Muñoz-Amatriaín, M., & Close, T.J. (2020). Genetic, textual, and archeological evidence of the historical global spread of cowpea (*Vigna unguiculata* [L.] Walp.). Legume Science, 2(4), e57.

Hiscox, J.D., & Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian journal of botany, 57(12), 1332-1334.

Hou, X., Fu, A., Garcia, V.J., Buchanan, B.B., & Luan, S. (2015). PSB27: A thylakoid protein enabling Arabidopsis to adapt to changing light intensity. Proceedings of the National Academy of Sciences, 112(5), 1613-1618.

Jiang, Y., Ding, X., Zhang, D., Deng, Q., Yu, C.L., Zhou, S., & Hui, D. (2017). Soil salinity increases the tolerance of excessive sulfur fumigation stress in tomato plants. Environmental and Experimental Botany, 133, 70-77.

Lal, H., Reddy, B. R., & Nath, V. (2018). Biometrical studies of yield and related traits in advance breeding lines of bush type vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. Legume Research-An International Journal, 41(6), 867-872.

Lal, H., Rai, N., & Nath, V. (2017). Biometrical approaches for selection of parents in vegetable cowpea (*Vigna unguiculata*) breeding programme. Indian Journal of Agricultural Sciences, 87 (8).

Legendre, R., Basinger, N.T., & Van Iersel, M.W. (2021). Low-cost chlorophyll fluorescence imaging for stress detection. Sensors, 21(6), 2055.

Lichtenthaler, H.K., Buschmann, C., & Knapp, M. (2005). How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R Fd of leaves with the PAM fluorometer. Photosynthetica, 43, 379-393.

Lichtenthaler, H.K., Langsdorf, G., Lenk, S., & Buschmann, C. (2005). Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. Photosynthetica, 43, 355-369.

Lu, Y. (2016). Identification and roles of photosystem II assembly, stability, and repair factors in Arabidopsis. Frontiers in plant science, 7, 168.

Mishev, K., Stefanov, D., Ananieva, K., Slavov, C., & Ananiev, E.D. (2009). Different effects of dark treatment on pigment composition and photosystem I and II activities in intact cotyledons and primary leaves of *Cucurbita pepo* (zucchini). Plant growth regulation, 58(1), 61-71.

Mussgnug, J.H., Thomas-Hall, S., Rupprecht, J., Foo, A., Klassen, V., McDowall, A., Schenk, P.M., Kruse, O., & Hankamer, B. (2007). Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. Plant biotechnology journal, 5(6), 802-814.

Omomowo, O.I., & Babalola, O.O. (2021). Constraints and prospects of improving cowpea productivity to ensure food, nutritional security and environmental sustainability. Frontiers in Plant Science, 12, 751731.

Prinzenberg, A.E., Víquez-Zamora, M., Harbinson, J., Lindhout, P., & van Heusden, S. (2018). Chlorophyll fluorescence imaging reveals genetic variation and loci for a photosynthetic trait in diploid potato. Physiologia Plantarum, 164(2), 163-175.

Qian, Y., Zhou, X., Han, L., Sun, Z., & Ju, G. (2011). Rapid light-response curves of PS II chlorophyll fluorescence parameters in the leaves of *Salix babylonica*, Salix'J172'and *Salix leucopithecia* to Cd2+ stress. Journal of Beijing Forestry University, 33(6), 8-14.

Rao, L., Li, S., & Cui, X. (2021). Leaf morphology and chlorophyll fluorescence characteristics of mulberry seedlings under waterlogging stress. Scientific Reports, 11(1), p.13379.

Rochaix, J.D. (2011). Regulation of photosynthetic electron transport. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1807(3), 375-383.

Rousseau, C., Hunault, G., Gaillard, S., Bourbeillon, J., Montiel, G., Simier, P., Campion, C., Jacques, M.A., Belin, E., & Boureau, T. (2015). Phenoplant: a web resource for the exploration of large chlorophyll fluorescence image datasets. Plant Methods, 11, 1-12.

Sánchez-Moreiras, A.M., Graña, E., Reigosa, M.J., & Araniti, F. (2020). Imaging of chlorophyll a fluorescence in natural compound-induced stress detection. Frontiers in plant science, 11, 583590.

Shi, Y., Chen, J., & Hou, X. (2020). Similarities and differences of photosynthesis establishment related mRNAs and novel lncRNAs in early seedlings (Coleoptile/Cotyledon vs. True Leaf) of rice and Arabidopsis. Frontiers in Genetics, 11, 565006.

Stirbet, A. (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. Journal of Photochemistry and Photobiology B: Biology, 104(1-2), 236-257.

Stirbet, A., & Govindjee. (2012). Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J–I–P rise. Photosynthesis research, 113, 15-61.

Tsai, Y.C., Chen, K.C., Cheng, T.S., Lee, C., Lin, S.H., & Tung, C.W. (2019). Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency. BMC plant biology, 19, 1-17.

Wang, Y., Jie, W., Peng, X., Hua, X., Yan, X., Zhou, Z., & Lin, J. (2019). Physiological adaptive strategies of oil seed crop *Ricinus communis* early seedlings (cotyledon vs. true leaf) under salt and alkali stresses: From the growth, photosynthesis and chlorophyll fluorescence. Frontiers in plant science, 9, 1939.

Xu, Q., Ma, X., Lv, T., Bai, M., Wang, Z., & Niu, J. (2020). Effects of water stress on fluorescence parameters and photosynthetic characteristics of drip irrigation in rice. Water, 12(1), 289.

Zhang, J.L., Wang, Y.Y., Sun, L.Q., Wei, T.T., Gu, X.H., Gao, F., & Li, X.D. (2013). Effects of paclobutrazol on the yield, quality, and related enzyme activities of different quality type peanut cultivars. Ying Yong Sheng tai xue bao= The Journal of Applied Ecology, 24(10), 2850-2856.

Zheng, W., Wang, P., Zhang, H., & Zhou, D. (2011). Photosynthetic Characteristics of the Cotyledon and First True Leaf of Castor (*Ricinus communis* L.). Australian Journal of Crop Science, 5(6), 702-708.

सारांश

लोबिया पानी की कमी वाले उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्रों में उगाया जाने वाला एक महत्वपूर्ण अनाज और दलहनी सब्जी फसल है। इन क्षेत्रों में लोबिया के ऐसे जीनोटाइप की आवश्यकता होती है जो अंकुरण अवस्था में तेजी से स्थापित हो सकें। लोबिया के अंकुरण के दौरान होने वाली घटना की बेहतर समझ हमें उन किस्मों को विकसित करने में मदद कर सकती है जिनमें मिट्टी में जल्दी स्थापित होने के गुण पाए जाते हैं। वर्तमान अध्ययन लोबिया अंकुरण के विभिन्न विकासात्मक चरणों में बीजपत्र क्लोरोफिल सामग्री और क्लोरोफिल प्रतिदीप्ति में घटनाप्रावस्था भिन्नता की जांच करने के लिए आयोजित किया गया था क्योंकि उपरोक्त दोनों प्रकाशसंश्लेषक क्षमता के संकेतक हैं और गैस विनिमय मापदंडों की तुलना में, ये प्रकृति में उच्च कार्यक्षमता, त्वरित और सरल हैं। लोबिया अंकुरण के तीन घटनाप्रावस्था चरणों में जिन्हें बंद, खुला और खुला प्राथमिक पत्ता (यूपीएल) बीजपत्र नाम दिया गया है उनमे कुल क्लोरोफिल सामग्री और क्लोरोफिल प्रतिदीप्ति मापदंडों जैसे अधिकतम प्रतिदीप्ति, पीएस II की अधिकतम क्वांटम दक्षता, पीएस II की संभावित प्रकाशसंश्लेषक सक्रियता, स्थिर-अवस्था आपेक्षिक प्रतिदीप्ति गिरावट अनुपात और लेक मॉडल के आधार पर प्रकाश रसायनिक शमन का स्थिर-अवस्था गुणांक को अंकुरित लोबिया के बीजपत्रों में मापा गया। इस अध्ययन मे यह पाया गया कि सभी लोबिया जीनो टाइप में बंद बीजपत्रों की तुलना में यूपीएल बीजपत्रों मे कुल क्लोरोफिल सामग्री, अधिकतम प्रतिदीप्ति, अधिकतम क्वांटम दक्षता, संभावित प्रकाशसंश्लेषक सक्रियता, स्थिर-अवस्था आपेक्षिक प्रतिदीप्ति गिरावट अनुपात, प्रकाश रसायनिक शमन का स्थिर-अवस्था गुणांक काफी अधिक है, जो कि प्रकाश संश्लेषक दक्षता के विश्वसनीय संकेतक हैं। इसके अलावा, लोबिया बीजपत्र में कुल क्लोरोफिल सामग्री की क्लोरोफिल प्रतिदीप्ति मापदंडों के साथ महत्वपूर्ण और सकारात्मक रूप से सह संबद्धता पाई गई। इस प्रकार वर्तमान अध्ययन से पता चलता है कि यूपीएल बीजपत्र में बेहतर विकसित प्रकाशसंश्लेषण तंत्र होता है और पौधे इस फेनोलॉजिकल चरण में अपने विकास के लिए बीजपत्र भंडारण से स्वतंत्र होते हैं। इसके अलावा यह भी स्पष्ट होता है कि आनुवंशिक आधार पर लोबिया बीजपत्र में क्लोरोफिल प्रतिदीप्ति मापदंडों की प्राकृतिक भिन्नता होती है जिसका उपयोग प्रारंभिक रूप से स्थापित लोबिया जीनोटाइप के प्रजनन और चयन कार्यक्रमों में किया जा सकता है जहां क्लोरोफिल प्रतिदीप्ति का उपयोग चयन उपकरण के रूप में किया जा सकता है।