Learning about chlorophyll and anthocyanins as potential indicators of plant physiological state

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Abstract: Currently, increased crop yield is direly needed considering the ever-increasing world population and climate change. Crops growth and production is mainly dependent upon their physiological state and vigor. Several forms of plant pigments exist naturally. They are categorized into four major groups: namely betalains (betacyanins, betaxanthins), carotenoids (carotenes, xanthophylls), chlorophylls (Chl a and b), and flavonoids (anthocyanins, aurones, chalcones, flavonols, proanthocyanidins). Out of all these, Chlorophylls (Chl) and Anthocyanins (Anth) are the two most important plant pigments that provide valuable insight into the plant physiological state. Primary function of Chl is the conversion of solar energy into chemical energy that is further utilized in the photosynthetic process, whereas Anth are multifunctional molecules that apart from coloration of plant organs also play an important role in stress mitigation. This study might serve as a guide for students interested to learn about plant physiology.

Keyword: Plant pigments

Aprender sobre la clorofila y las antocianinas como posibles indicadores del estado fisiológico de las plantas

Resumen: En la actualidad, el aumento de la producción de los cultivos es una necesidad imperiosa, teniendo en cuenta el incremento de la población mundial y el cambio climático. La producción y el desarrollo de los cultivos dependen principalmente de su estado fisiológico y su vigor. Existen varias formas de pigmentos vegetales en la naturaleza. Normalmente, se clasifican en cuatro grandes grupos: betalaínas (betacianinas, betaxantinas), carotenoides (carotenos, xantofilas), clorofilas (Chl a y b) y flavonoides (antocianinas, auronas, chalconas, flavonoles, proantocianidinas). De todos ellos, las clorofilas (Chl) y las antocianinas (Anth) son los dos pigmentos vegetales más importantes que proporcionan una valiosa información sobre el estado fisiológico de la planta. La función principal de las Chl es la conversión de la energía solar en energía química que se utiliza posteriormente en el proceso fotosintético, mientras que las Anth son moléculas multifuncionales que, además de la coloración de los órganos de la planta, desempeñan un importante papel en la mitigación del estrés. Este estudio podría servir de guía para los estudiantes interesados en aprender sobre fisiología vegetal.

Palabra clave: Pigmentos de las plantas

Introduction

Agriculture sector is facing several challenges including increasing world population and climate change. Higher crop yields are required to ensure food safety and security in such challenging circumstances (Martos et al., 2021). Crops growth and yield is mainly dependent upon their physiological state and vigor. The suboptimal growth conditions negatively impact crop yield. Whereas the optimal growth conditions and stress mitigation can effectively contribute for an enhanced crop yield. Plant physiological status can be assessed using a variety of biochemical and molecular techniques. In this regard, two chief plant pigments, namely chlorophyll (Chl) and anthocyanins (Anth), hold a prominent stance (Gitelson et al., 2003). Assessment of these plant pigments provides useful information about the physiological status of plants (Gitelson et al., 2009).

Chl is a natural plant pigment that mostly occurs in plant leaves and facilitates the photosynthetic process. The name "Chlorophyll" for these pigments was first used by Pelletier and Caventou in 1818 (Humphrey, 1980). Since then, a plethora of studies have been done about the possible role of Chl in plants. Primary function of Chl is the conversion of solar energy into chemical energy that is further utilized in the photosynthetic process (Gitelson et al., 2003). It has been well established that foliar Chl concentration is related to photosynthetic potential, which means more the photosynthetic pigments, more the absorption of solar radiation (Garriga et al., 2014). Although this might be true in ideal conditions but during a stress condition this might not be true. Similarly, Chl concentration also provides useful insights for total leaf nitrogen and phytonutrients. Under stress conditions plants exhibit varied Chl concentrations and thus altered photosynthetic potential (Garriga et al., 2014). Therefore, an evaluation of these parameters can certainly indicate the physiological status of plants.

Anth are another important water-soluble flavonoid plant pigments. Scientific research on Anth began in 1920s by Sir R. Robinson (Robinson & Robinson, 1931). Previously, Anth were associated with fruits and flowers coloration in higher plants. However, recent studies have revealed their contributions in plant defense mechanisms, ecophysiology, and propagation (Santos-Buelga et al., 2014). Role of Anth against heavy metals, UV-B, protection against photoinhibition, scavenging of reactive oxygen species (ROS), and drought stress mitigation in plants have also been reported previously (Blando et al., 2004; Gould, 2004; Gould et al., 2000; Merzlyak et al., 2008). Under stress, Anth content and synthesis is reported to have been increased (Garriga et al., 2014). Considering these studies, Anth content determination provides a significant information about plant's physiological state.

This study presents useful insights about the determination of plant physiological status and vigor through the determination of Chl and Anth content in plant leaves. The subject matter is further elaborated with some examples in the light of previously reported studies. The present study is meant to facilitate the students in understanding the basics of plant physiology.

Plant Pigments

Several forms of plant pigments exist naturally. For instance, more than 700 flavonoids and over 600 carotenoids structures have been reported previously. Plant pigments, based on their biosynthesis and structure, are categorized into four major groups (Figure 1): namely betalains (betacyanins, betaxanthins), carotenoids (carotenes, xanthophylls), chlorophylls (*Chl a* and *b*), and flavonoids (anthocyanins, aurones, chalcones, flavonols, proanthocyanidins) (Davies, 2009). Following are the two most prominent plant pigments.

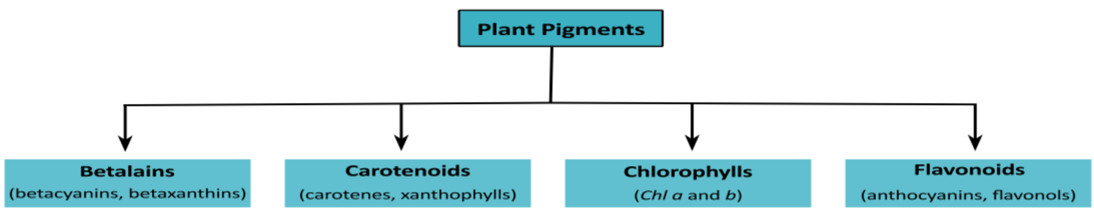

Figure 1. Four major classes of plant pigments.

1. Chlorophylls (Chl)

The word Chlorophyll is derived from Greek words '*chloros'* that means green and '*phyllon'* that means leaf. They are made up of carbon, nitrogen, and centrally held magnesium ions (Pareek et al., 2017). The basic unit of Chl is porphyrin, and chlorin, that creates cyclic forms giving rise to isocyclic ring held by –CH connections. These cyclic forms of porphyrin, also termed as tetrapyrroles, have a central magnesium ion. Furthermore, Chl contain phytol that renders them hydrophobic molecules (Scheer, 2006). Chl, the widespread plant pigments, are cyclic tetrapyrrole compounds found in various plant tissues including flowers, fruits, and leaves (Davies, 2009). Although, chloroplasts are the principal plant organelles that contain the highest amount of Chl (Scheer, 2006).

They are primarily responsible for light absorption and its subsequent conversion into chemical energy through the process of photosynthesis. Chl also drive the fixation of carbon dioxide (CO_2) into carbohydrates and energy by the absorption of solar light (Pareek et al., 2017). Chl pigments are capable of red and blue light at 430 and 660 nm respectively and have a green spectrum. *Chl a* and *Chl b* are the most important Chl with respect to plant photosystems. Although, other classes of Chl including *c*, *d*, and *f* also exist in nature but in various photosynthetic organisms (Scheer, 2006).

Generally, the incident solar light on plant leaf encounters three fates i.e., part of the incident light is absorbed by the Chl, part of it is reflected, and the part of it is dissipated as heat (Figure 2). The absorbed light is used for the excitation of electrons that subsequently generates energy molecules including ATP. The reflected light that is not absorbed by Chl is termed as fluorescence. Only a small proportion, only 1 or 2%, of total incident light is absorbed by the plant and the rest is re-emitted as fluorescence and heat (Maxwell & Johnson, 2000; Pareek et al., 2017). Therefore, measurement of Chl fluorescence has become very common practice in today's plant ecophysiology studies, as it indicates the status of plant physiological status i.e., healthier the plant lower the fluorescence. This phenomenon of fluorescence is mostly related to *Chl a*. Several studies have been reported that have used the variation of *Chl a* fluorescence for monitoring plant stresses including drought stress, heat stress, environmental pollution, photoinhibition, UV stress, water quality, and salt stress (Kalaji et al., 2017).

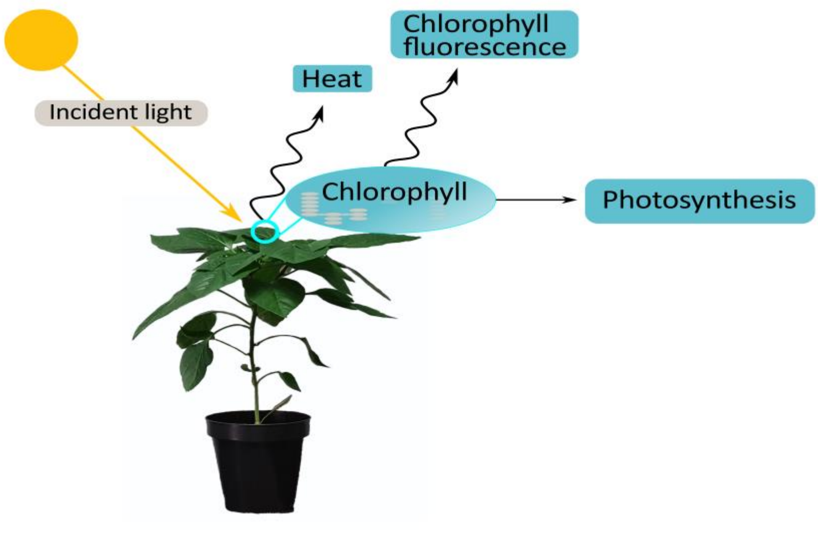

Figure 2. The incident solar light on plant leaf. One part of this light is absorbed by chlorophyll for its use in photosynthesis process, other part is reflected as heat, while the remaining part is emitted as fluorescence.

2. Anthocyanins (Anth)

The word anthocyanin is derived from the Greek words '*anthos'* and '*kyanos'* that mean flower and blue respectively. Anth impart colors to plants and are also consumed in human diet given their potential as antioxidants, antimicrobial, antitumor, antidiabetic, and anti-obesity agents (Blando et al., 2004; Garriga et al., 2014; Turturică et al., 2015). Anth are flavonoids that exhibit a basic structure of C6–C3–C6. Anth can be found in the sub-epidermal cells of leaves, and in the epidermal cells of petals. Although they can be synthesized in roots, stems, bracts, and trichomes (Davies et al., 2018; Gould et al., 2000). Usually, Anth accumulate in the vacuole but they can also be find in anthocyanoplasts (Strack & Wray, 2017).

Anth in plants are mainly responsible for coloration in plants, especially in fruits and flowers, that leads to attracting pollinators. Besides they are also reported to provide protection against photoinhibition and stress (Garriga et al., 2014; Gould et al., 2000). Anth are susceptible to pH, metal ions, enzymes, light, oxygen, sugars, temperature, etc. and can alter the color under such conditions. Anth can be produced by plants in response to biotic or abiotic stress. For instance, cold or nutrient deficiency induced leaf margins pigmentation by the accumulation of Anth (Albert et al., 2015; TURTURICĂ et al., 2015). Anth are reported to protect chloroplasts, and indirectly to Chl, by the absorption of extra light, thereby safeguarding them from photoinhibition and photooxidation (Gould, 2004).

Plant vigor based on chlorophyll and anthocyanins

Plant Chl content is affected by changes in the environment and even by ontogenic changes. For instances, Chl degradation occurs at the germination of seeds, maturation of inflorescence, fruit ripening, and premature death. Similarly, in case of biotic (disease, grazing, harvesting, etc.) and abiotic (temperature, UV rays, salinity, etc.) stress plant undergoes Chl degradation on a whole plant level (Scheer, 2006). Therefore, this can be inferred that if plant is under some stress or experiences mineral deficiencies it would have drastically lower Chl content as compared to a normal plant. Similarly, the lower content of Chl would also affect the photosynthetic potential of plant and the stressed plant would exhibit higher levels of fluorescence. A graphical representation of Anth and Chl contents in a healthy and stressed plant is presented in Figure 3. Following are the few examples of stressful environments which indicate the physiological status of plants in terms of their Chl content and fluorescence.

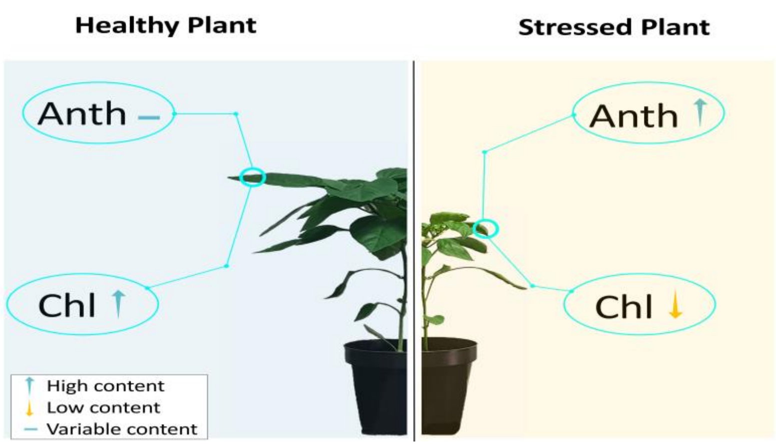

Figure 3. Illustration of anthocyanins and chlorophyll contents in a healthy and stressed plant. Where, Chl represent chlorophyll and Anth represent anthocyanins.

Solanum melongena L. plants have been found to exhibit decreased Chl content when subjected to moisture stress (Prakash & Ramachandran, 2000). Similarly, increased *Chl a* fluorescence and decreased Chl content was reported in *Olea europaea* cv. Dezful under water stress (Khaleghi et al., 2012). Similarly, several other studies have also reported in the reduction of Chl content under salt, temperature, UV-B, drought, and light stresses in peppers, wheat, tomatoes, maize, and strawberry plants (Bhandari et al., 2018; Gholamin & Khayatnezhad, 2020; Khayatnezhad & Gholamin, 2021; León-Chan et al., 2017; Park et al., 2018; Taïbi et al., 2016; Tang et al., 2020).

Similarly, many recent studies have demonstrated the accumulation of Anth in different plant parts under biotic and abiotic stress help mitigate its effects (Naing & Kim, 2021). Therefore, it can be inferred that plants under stress would have different content of Anth as compared to the plants grown in normal conditions, although plants ontogenic (development) stage should also be considered. Consequently, this indicates physiological status of plant. Following are the few examples of stressful environments which indicate the physiological status of plants in terms of their Anth content.

Arabidopsis thaliana plants were found to mitigate the salt, cold, and drought stress by accumulation of Anth (Li et al., 2017). Likewise, increased accumulation of Anth in *Acmena acuminatissima*, *A. thaliana*, *Solanum tuberosum*, *Craterostigma wilmsii*, and *Xerophyta viscosa* have been reported under light, heavy metals, temperature, nitrogen, salt, and drought stress (Ai et al., 2018; Cheng et al., 2013; Liang & He, 2018; Naing et al., 2017; Wang et al., 2016; Zhu et al., 2018).

Conclusion

Chl and Anth are two most important plant pigments and play their role, among others, in harvesting light energy and coloration of different plant parts. Under biotic or abiotic stress contents of these pigments fluctuate in order to mitigate the stress. An assessment of only these two parameters can effectively indicate the physiological status of plant and the stressed plants can be distinguished easily from healthier ones. This study might serve as a guide for students interested to learn about plant physiology.

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