

## Dicamba+2, 4-D affects the shape of the Kautsky curves in wild mustard (*Sinapis arvensis*)

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### Abstract

Measuring chlorophyll fluorescence is a noninvasive, highly sensitive and fast way to give important information about the photosynthetic apparatus of plants. Applications of five doses (73.3, 110, 165, 247.5, 371.2 g ai ha<sup>-1</sup>) of the post emergent broad leaf herbicide Dicamba+2, 4-D were tested on greenhouse-grown wild mustard (*Sinapis Arvensis*). Results showed that herbicide application changed the shape of the chlorophyll fluorescence induction curve (Kautsky curve) two days after spraying (DAS). Fluorescence parameters such as  $F_v/F_m$ , maximum quantum efficiency of PS II and  $F_{vj}$ , variable fluorescence at the J step were significantly reduced at the recommended dose (371.2 g. ai. ha<sup>-1</sup>).  $F_{vj}$  was determined as a rather stable parameter compared to the  $F_v/F_m$ . At dose of 371.2 g. ai. ha<sup>-1</sup>, the Kautsky curves were almost turned to the straight lines, demonstrating serious damage to plant photosynthesis apparatus. These results demonstrate the possibility of measuring chlorophyll fluorescence as an easy, fast and cost effective method, to study the efficacy of auxin type herbicides.

**Keywords:**  $F_v/F_m$ ,  $F_{vj}$ , fluorescence, dicamba+2, 4-D.

**Abbreviations:**  $F_v/F_m$ - Maximum quantum efficiency of PSII;  $F_m$ - Maximum fluorescence;  $F_0$ - Minimum fluorescence;  $F_j$ - Fluorescence at J step; ;  $F_v$ - Variable fluorescence;  $F_{vj}$ - Variable fluorescence at J step.

### Introduction

Chlorophyll *a* fluorescence induction kinetics is a highly sensitive and non-destructive method to study the photosynthetic electron transfer chain from PS II to PS I (Tongra et al., 2011). Light energy from the sun captured by plants' light harvesting complex that is not used in photochemistry reactions is released as heat and fluorescence. There are three processes (photochemical reaction, heat and fluorescence) that occur in competition with each other and any increase in the efficiency of one will cause decreases in yields of the other two (Maxwell and Johnson, 2000). The wavelength of fluorescence is longer than that of absorbed light. Thus, fluorescence yield can be quantified by exposing a leaf to light of a defined wavelength and then comparing it with the measurement of the re-emitted light that has a longer wavelength (Maxwell and Johnson, 2000). The total amount of chlorophyll fluorescence is small (1-2% of absorbed light) (Maxwell and Johnson, 2000). Illumination with 650 nm wavelength of healthy dark-adapted leaves provides a rise in amounts of chlorophyll fluorescence emission, with some characteristic phases. There are three phases based on the O, J, I, and P steps. These phases primarily indicate photochemical events related to PSII (Govindjee, 1995). The three phases are defined as follows: at the O-J phase complete reduction of the primary electron acceptor  $Q_A$  of PSII takes place within 50  $\mu$ s to 2 ms, the J-I phase corresponds to electron transfer from  $Q_A$  to  $Q_B$  that occurs between 2 to 30 ms and the I-P phase

corresponds to the release of fluorescence quenching by the oxidized plastoquinone pool that takes place within 30-500 ms (Strasser and Stirbet, 2001; Force et al., 2003) (Fig 1). Illumination of dark-adapted leaves produces a rise in fluorescence from a ground state ( $F_0$ ) at the O step to its maximum value ( $F_m$ ) at the P step within 1 second. Under this condition,  $Q_A$  is completely reduced and the value of  $F_v/F_m$  can then be determined, this value in all plants, independent of species, is approximately equal to 0.83 (Strasser and Stirbet, 2001; Appenroth et al., 2000).

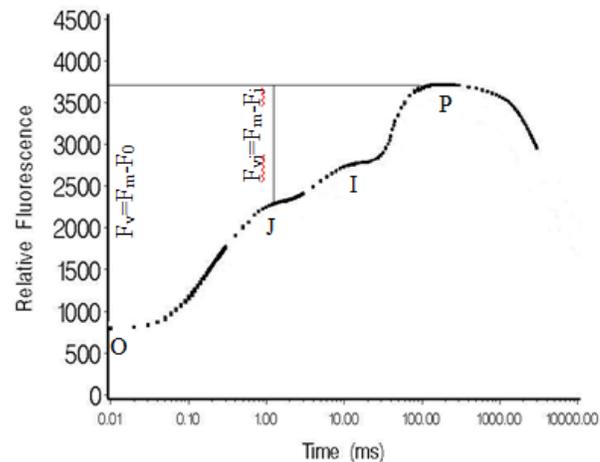
Fluorescence induction kinetics can be altered by many inhibitors of metabolic processes that are not directly involved in photosynthesis (Blowers, 1989; Percival and Baker, 1991; Crudace, 2000). The shape of the Kautsky curve and derived fluorescence parameters of *Arabidopsis* seedlings affected by herbicides (Asulam, Bifenox, 2,4-D, Diclofop-methyl, Glyphosate, and Imazapyr), which do not have a direct impact on photosynthesis (Barbagallo et al., 2003). Dicamba+2, 4-D (Dialen super<sup>®</sup>) is a selective post emergence root and foliar absorbed auxin type herbicide, which is rapidly translocated apo-simplastic throughout the plant. It controls annual, biannual and perennial broadleaved weeds in cereals, maize and pasture. Adventitious root formation at stem nodes is due to cell division and differentiation in the cambium, general tissue swelling and stem, petiole and leaf epinasty are visible symptoms that commonly follow auxin type herbicide

treatments (Cobb and Reade, 2010). The objective of this study was to determine whether or not dicamba+2, 4-D, an auxin type herbicide affects the chlorophyll fluorescence parameters in *S. arvensis*.

## Results and discussion

### The Kautsky curves

Dicamba+2, 4-D affected the shape of the Kautsky curves significantly at 2 DAS at all doses (Fig 2), although the onset of these changes was observed at 1 DAS (data not shown). Kautsky curves with distinctive OJIP steps in a healthy control *S. arvensis* were clearly observed, while these steps were eliminated in sprayed leaves (Fig 2). The OI phase corresponds to electron transfer from  $Q_A$  to  $Q_B$ , and fluorescence quenching during this step is controlled by PSII donor side, the IP step corresponds to fluorescence quenching by the oxidized plastoquinone pool (Strasser and Stirbet, 2001; Force et al., 2003). By increasing the dose of the dicamba+2, 4-D OJIP steps of the Kautsky curve were omitted; at the recommended dose ( $371.2 \text{ g. ai. ha}^{-1}$ ) the Kautsky curves turned in to a approximately straight lines (Fig 2). Each step of the Kautsky curve represents those photochemical events associated with PSII and a straight line represents a loss of OJIP steps indicating serious damage to the electron transfer chain in photosynthesis. Dicamba+2, 4-D is categorized as an auxin type herbicide. It causes inhibited plant growth and stimulates a concentration-dependent manner and different plant tissues showing differential sensitivity to applied exogenous auxins (Cobb and Reade, 2010). Growth inhibition by auxins is largely due to auxin induced ethylene evolution (Cobb and Reade, 2010). Furthermore, ethylene elicits biosynthesis of the hormone abscisic acid (ABA) (Grossmann et al., 2001). ABA induces stomatal closure (Cobb and Reade, 2010) and leads to a restriction of  $\text{CO}_2$  diffusion through stomata that appears to be responsible for a reduction of  $\text{CO}_2$  uptake (Cronic, 2000). Consequently, limited  $\text{CO}_2$  fixation leads to the accumulation of reactive oxygen species (ROS) such as  $\text{H}_2\text{O}_2$  that arises from increased thylakoid membrane electron leakage to  $\text{O}_2$  in the chloroplast (Dat et al., 2000).  $\text{H}_2\text{O}_2$  and super oxide radicals form hydroxyl radicals that result in lipid peroxidation and oxidative damage (Dat et al., 2000). It seems thylakoid membrane electron leakage and oxidative damage of thylakoid phospholipid membrane of the chloroplasts interrupts the electron transport chain (Z scheme) from PSII to PSI and this interruption of the Z scheme will change the Kautsky curve. Herbicides generating ROS and that cause peroxidation of the membrane lipid affect the stability of the photosynthetic apparatus and could contribute to change the shape of the chlorophyll fluorescence induction curve (Dayan and Zaccaro, 2012). The Kautsky curves measured in *S. arvensis* sprayed at the recommended dose of ( $371.2 \text{ g. ai. ha}^{-1}$ ) formed a straight line with no decay after the P step. This lack of fluorescence decay after 1000 ms (P step) suggests that PSII is inactivated although the dicamba+2, 4-D is not a PSII inhibitor. But at doses lower than those recommended, quenching of the curves occurs after the P step. Christensen et al., (2003) reported that bentazon, a PSII inhibitor, completely inhibited the PSII reaction centers and therefore quenching of the Kautsky curves after the P step did not occur. The research also proposed that unlike bentazon, the level of fluorescence quenching for

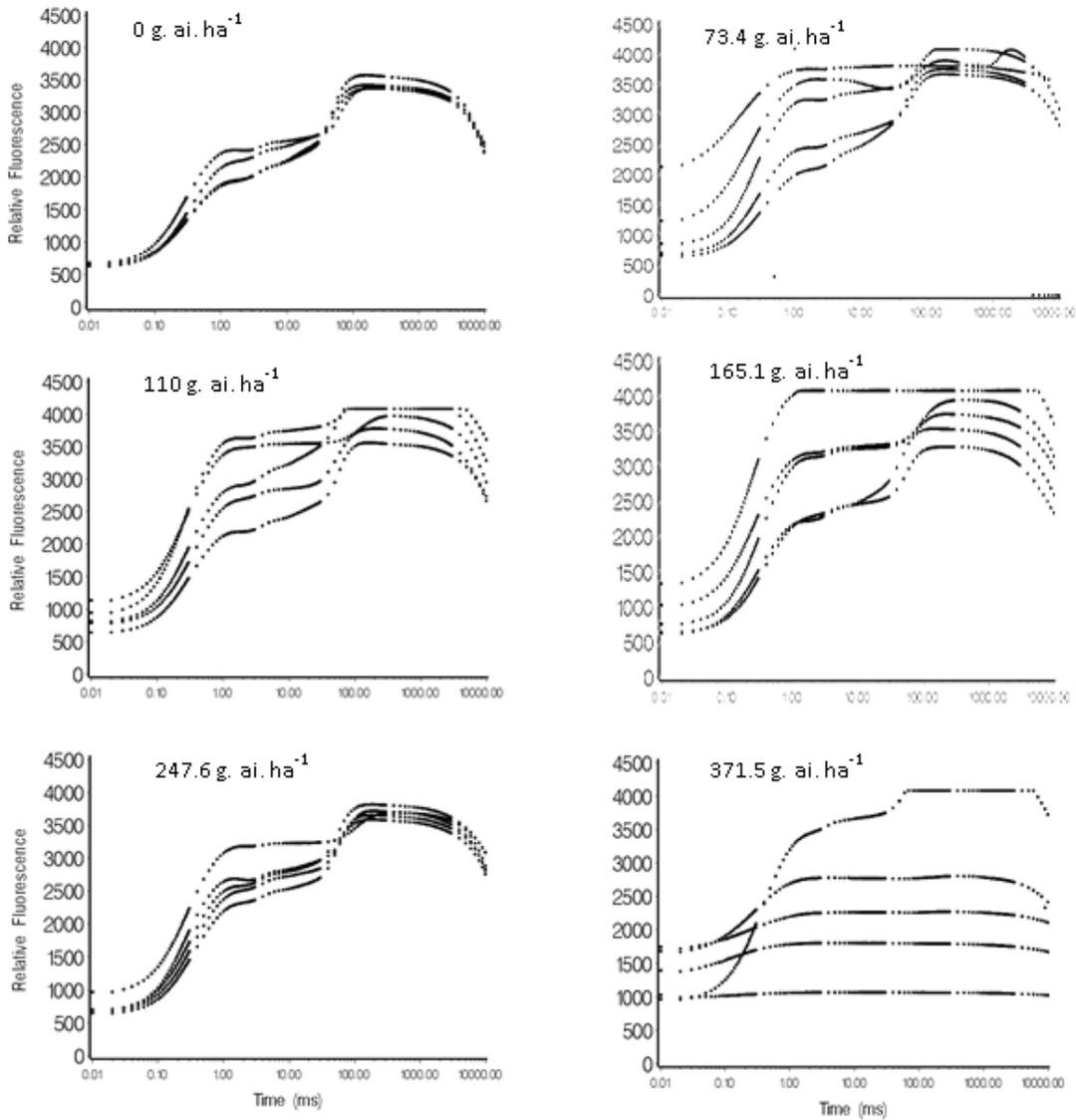


**Fig 1.** Kautsky curve recorded with Handy PEA instrument in a 30 minutes dark adapted leaf. The Kautsky curve rise from O to P levels is characterized by the OJIP steps reflecting PSII electron transport from water to PQ pool.

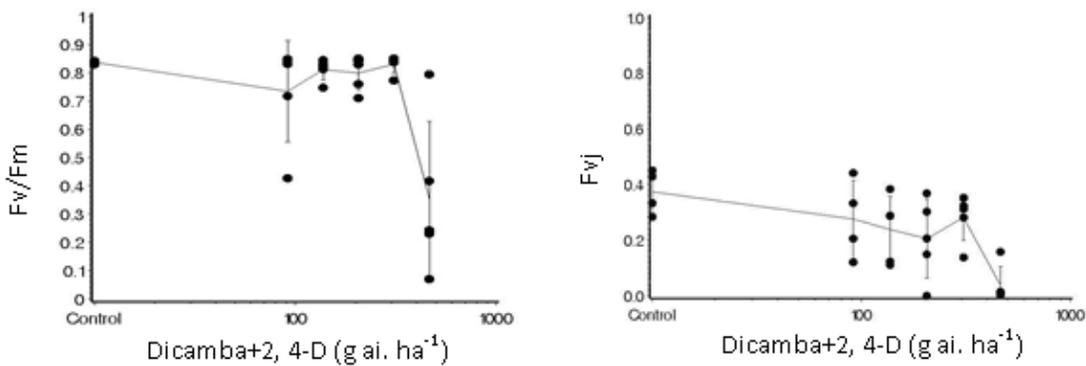
fluorochloridone, a phytoene desaturase inhibitor, and glyphosate, an EPSP synthase inhibitor at the P step suggests that at least some of the PSII reaction centers remains active. Abbaspoor and Streibig (2005) reported fluorescence quenching after the P step in oat leaves treated with clodinafop.

### The fluorescence parameters

To explain changes in the shape of the Kautsky curves in Fig 2 some important fluorescence parameters such as  $F_v/F_m$  and  $F_{vj}$  were plotted against different doses of dicamba+2, 4-D for 2 DAS and are shown in Fig 3.  $F_v/F_m$  and  $F_{vj}$  decreased as the dose was increased (Fig 3). The value of the parameter  $F_v/F_m$  in all plants independent of species is approximately 0.83 (Strasser and Stirbet, 2001; Appenroth et al., 2000) but it reduces under stress conditions. In an untreated control (Fig 3) the value of  $F_v/F_m$  was 0.8 but with increasing the dose this value started to decrease. As shown in Fig 3.,  $F_{vj}$  started to decline at much lower doses than  $F_v/F_m$ , identifying it as a good and sensitive indicator of the condition of a plant's health and PS II functionality. Abbaspoor and Streibig (2007) also reported  $F_{vj}$  and the area between the Kautsky curve and the maximum fluorescence were much more sensitive than  $F_v/F_m$  measured in black nightshade and sugar beet sprayed by desmedipham, phenmedipham and a mix of the two. As electron transfer reactions frequently generate reactive and harmful intermediates as by-products, these processes take place in the lipid bilayers to inhibit direct contact with water or high concentrations of oxygen and cells are encircled with numerous antioxidants and ROS-scavenging enzyme cycles that in healthy plants, with no biotic or abiotic stresses, serve to extinguish excess production of reactive oxygen species (Mittler, 2002). However, plastids have complex membrane systems consisting of outer and inner envelopes where important processes such as the synthesis of glycerolipids, pigments (chlorophylls, carotenoids), and prenylquinones (plastoquinone and  $\alpha$ -tocopherol) take place (Joyard et al., 1998). The presence of at least 700 different proteins demonstrates that a high level of physiological activity is going on in plastids (Newmeyer and



**Fig 2.** Effect of different doses of dicamba+2, 4-D on the shape of the Kautsky curves (10 s) at *S. arvensis* 2 days after spraying. Each curve presenting one replication. Changes at the shape of the Kautsky curve compare to the standard shape of healthy *S.arvensis* (0 g. ai. ha<sup>-1</sup>) shows that PSII is damaged.



**Fig 3.** Effect of dicamba+2, 4-D doses on chlorophyll fluorescence parameters at 2 days after spraying. Each point presenting one replication. Decreasing of these two parameters shows that plants photosynthesis is not working properly.

Ferguson-Miller, 2003). Considering the importance of phospholipid bilayers in accomplishing different physiological processes and specially the role of the thylakoid membrane at electron transfer, any damage either directly or indirectly interrupts electron transfer systems and photosynthesis. The over production of H<sub>2</sub>O<sub>2</sub> is a consequence of auxin type herbicides that leads to phospholipid membrane destruction (Grossmann et al., 2001); this may be the cause of the change in shape of the Kautsky curves.

## Materials and methods

### Plant material

*S. arvensis* seeds were obtained from Gorgan, Golestan province, Iran. After breaking dormancy of *S. arvensis* seeds (by keeping seeds for one week at 5°C and then for two days at 25°C), seeds were transferred to trays containing peat and moss at the ratio of 1:1. After germination, seedlings were transplanted into 12-cm diameter pots filled with a mixture of soil, sand and peat (1:1:1 v/v). The pots were sub-irrigated daily. The average temperature and humidity in the green house were 23°C and 75% respectively.

### Herbicide application

Treatments consisted of five doses of dicamba+2, 4-D (73.3, 110, 165, 247.5, 371.2 g. ai. ha<sup>-1</sup>) plus a control. *S. arvensis* plants were sprayed at the three-leaf stage with 200 L ha<sup>-1</sup> of spray solution at a pressure of 300 kPa using a flat fan 8001 nozzle.

### Fluorescence measurement

The experiment was set up as a completely randomized design with five replications for each treatment, and done in a greenhouse in Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2011. Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (Handy-PEA, Hansatech Instruments, King's Lynn, Norfolk, UK) which emits a light of 650 nm wave length with an intensity of 3000 μmol photons m<sup>-2</sup> s<sup>-1</sup> for 10 seconds on dark-adapted leaves (30 minutes dark adapted by covering the leaves with a clip). Kautsky curves and their parameters, obtained by the BioLyzer program (Rodriguez and Strasser, 2002) were further analyzed by SAS software (9.2). The parameters analyzed in this experiment were  $F_v/F_m = (F_m - F_0)/F_m$  and  $F_{vj} = (F_m - F_j)/F_m$ , where F<sub>m</sub>: maximum fluorescence, F<sub>0</sub>: ground state fluorescence and F<sub>j</sub>: Fluorescence at J step (Fig 1).

## Conclusion

The effects of applications of Dicamba+2, 4-D on the Kautsky curve, indirectly gives a chance to guess which part of the PSII is damaged by the herbicide. Even the Kautsky curve shape after 1000 ms (end of fast phase of fluorescence and beginning of the slow phase) could be used as an index to determine the power of a herbicide to destroy PSII. Results of these tests demonstrate that chlorophyll fluorescence can also be used as a good marker to identify the efficacy of auxin type herbicides such as dicamba+2, 4-D as well as PS II inhibitors and ACCase inhibitors.

Finally it may be suggested, that in order to determine the various effects of different herbicide families in terms of modes of action on fluorescence parameters of problematic weeds; that more experiments are done to determine their individual effects on the shape of the Kautsky curve and fluorescence traits and to identify fluorescence parameters that are more capable of describing herbicidal effects of a specific herbicide family.

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