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Photosynthetic gas exchange and chlorophyll fluorescence of female hemp plants during sexual reversal treatments

[Intercambio gaseoso y fluorescencia de la clorofila en plantas femeninas de Cannabis durante tratamientos de reversión sexual]

Henry Andrés Mejía-Londoño, Julián David Otálvaro-Gutiérrez, Carlos Felipe Barrera-Sánchez & Oscar de Jesús Córdoba-Gaona

Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Sede Medellín, Colombia

Reviewed by:
Patricia Peralta
Instituto Nacional de Tecnología Agropecuaria
Argentina

Alberto González
Universidad de Santiago de Chile
Chile

Correspondence:
Henry Andrés **MEJÍA-LONDOÑO**
hmejial@unal.edu.co

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Abstract: Sexual reversal methods are commonly used in plant breeding programs, allowing male flowers from female plants or vice versa. This work evaluated sexual reversal methods in female Cannabis plants and their effect on gas exchange activity. Plants treated with 1, methyl-cyclopropene (1-MCP), and aminoetoxyvinylglycine (AVG) showed differences in net photosynthesis (A) and stomatal conductance (gs) between the periods before and after sexual reversal treatments. Quantum yield (Qy), electron transport rate (ETR), and non-photochemical quenching (NPQ) did not show a relationship to the treatments, an increase in Qy and ETR, and a reduction in NPQ were observed after applying treatments. 1-MCP, AVG, and STS (silver thiosulfate) were effective in sexual reversal, while photoperiod changes did not induce the formation of male flowers. Induction of sexual reversion in Cannabis plants did not generate variations in energy dissipation mechanisms through photosystems.

Keywords: *Cannabis sativa* L.; Photosynthesis; Silver thiosulfate; Qy; AVG

Resumen: Los métodos de reversión sexual se utilizan comúnmente en los programas de fitomejoramiento, permitiendo la formación de flores masculinas a partir de plantas femeninas y viceversa. Este trabajo tuvo como objetivo evaluar métodos de reversión sexual en plantas femeninas de Cannabis y su efecto sobre el intercambio de gases. Plantas tratadas con 1-metil-ciclopropano (1-MCP) y aminoetoxivinilglicina (AVG) mostraron diferencias en fotosíntesis neta (A) y conductancia estomática (gs) entre los periodos antes y después de los tratamientos de reversión sexual. El rendimiento cuántico (Qy), la tasa de transporte de electrones (ETR) y la disipación no fotoquímica (NPQ) no mostraron relación con los tratamientos, se observó un incremento en Qy y ETR y una reducción en NPQ después de la aplicación de los tratamientos. 1-MCP, AVG y STS (tiosulfato de plata) fueron efectivos en la reversión sexual, mientras que los cambios en el fotoperiodo no indujeron la formación de flores masculinas. La inducción de la reversión sexual en plantas de Cannabis no generó variaciones en los mecanismos que disipan la energía a través de los fotosistemas.

Palabras clave: *Cannabis sativa* L.; Fotosíntesis; Tiosulfato de plata; Qy; AVG

INTRODUCTION

Hemp, *Cannabis sativa* L. (Cannabaceae) is an ancestral species whose origin extends from Europe to Asia. Various uses vary in medicine, fiber production, and ritual ceremonies (Grotenhermen, 2006; Clarke & Merlin, 2013; Bonini *et al*., 2018). For medical uses, the interest in hemp compounds (canmononabidiol – CBD) has increased. It can be delivered in various ways (inhaled, swallowed, or topical application to the skin or buccal mucosa) (Ebbert *et al.,* 2018). Cannabis or cannabinoids indications are effective for the Oncology (metastatic or chemotherapy-related symptoms), Neurology (muscle spasticity syndromes in multiple sclerosis), and Psychiatry (posttraumatic stress disorder) treatment, as well as for the management of chronic pain in adults, Alzheimer and Crohn disease, glaucoma, hepatitis C infection, and AIDS (Ablin *et al*., 2016; Ebbert *et al*., 2018).

Hemp is a dioecious species with sexual dimorphism. This characteristic hinders sex determination in the early stages of development, especially in plants propagated by sexual seeds. The expression and differentiation of flowering only occur when the plants have reached the fourth node stage, where the meristem primordia produced could, in most cases, develop into an inflorescence bud (Truta *et al*., 2002; Moliterni *et al.,* 2004). Although monoecious plants may occur occasionally, the sexual phenotype of *Cannabis* often shows some differentiation of hermaphrodite flowers or bisexual inflorescences. This, because of intensive breeding programs (Truta *et al*., 2002; Moliterni *et al*., 2004; Hobza *et al*., 2018).

Currently, monoecious forms (female plants) are required because female inflorescences accumulate significantly higher concentrations of CBD per unit of biomass; however, seed production also involves developing male flowers (Razumova *et al*., 2015; Lubell & Brand, 2018). According to Mansouri *et al*. (2013), female inflorescences accumulate 3.1 times higher concentrations of CBD than male flowers. While at the foliar level, female and male plants do not show differences in phytocannabinoid concentrations.

The reversion of dioecious to monoecious flowers in hemp plants convert this species into a kind of interest for a genetic breeding program; The sexual reversal of dioecious plants has been reached, through hormonal manipulation, especially ethylene inhibitors, as Chailakhyan & Khryanin have demonstrated since 1978. The masculinizing effect has been reported in some chemical compounds such as silver thiosulfate (STS), aminoetoxyvinylglycine (AVG), and silver nitrate ($AgNO_3$). They are commonly used in the hemp industry to produce male flowers on genetically female plants, while precursors or activators of the ethylene biosynthesis, like Ethephon, have a feminizing effect (Hall *et al*., 2012). STS and 1, methyl-cyclopropene (1-MCP) are ethylene action inhibitors, while Aminoethoxy-vinyl-glycine (AVG) inhibits the conversion of methionine to ethylene (Ram & Sett, 1982a; Ram & Sett, 1982b). Application of 3 mM of STS promoted the transformation of female to male flowers if plants grow under short-day conditions (Lubell & Brand, 2018), while 75 µg of $AgNO_3$ were necessary to inhibit the action of ethylene for producing male flowers only (Ram & Sett, 1982c). Leaf apical application of AVG to female hemp plants induced fertile male flowers, doses of 1 µg $plant^{-1}$ were ineffective, and 75 µg $plant^{-1}$ formed only male flowers (Ram & Sett, 1982a).

On the other hand, in addition to the growth regulators, photosynthesis and the fluorescence chlorophyll parameters have been related to the different processes of floral development (Heslop-Harrison, 2018). External and internal factors can simultaneously regulate photosynthesis. External factors (light, water, nutrients, heat, and carbon dioxide) directly affect photosynthesis when they alter the rates of chemical processes in the photosynthetic pathway. On the other hand, internal factors as membrane and genetic regulation, CO_2 and O_2 concentration, stomatal and mesophilic conductance are endogenous regulatory elements of photosynthesis (Teskey *et al*., 1995; Duca, 2015). The floral induction generates changes in the photosynthetic activity of plants by increasing the levels of endogenous sucrose in leaves and tips. Nevertheless, photosynthetic system and primary metabolites participation in hemp flowering initiation processes is not clearly understood, much less is its relation to sexual reversion (Chandler, 2011; Cho *et al.*, 2018).

It is still unclear how photosynthetic interact during light, thermal and hormonal induction, and other flowering initiation processes (Samuolienė & Duchovskis, 2012). This work aimed to know if the photosynthetic activity and the chlorophyll fluorescence in female *Cannabis* plants can be used as a physiological indicator of sexual reversal by the activity of chemical substances that inhibit the action of ethylene.

MATERIALS AND METHODS

Location

The study was conducted in the Breeder S.A.S. company located in Bello, Antioquia, Colombia, in a plastic house with covers of high-density polyethylene (75% transmissivity) and natural ventilation from November 2019 to February 2020. Breeders S.A.S. is registered as a research unit in psychoactive cannabis breeding through the Colombian Agricultural Institute (ICA) resolution No. 00030034 of August 14, 2018.

Plant material

A dioecious female hemp cultivar with different origins was used in this study. Cuttings from female mother plants were propagated in germination chambers for 17 days with controlled: temperature (25 to 30°C), relative humidity (≥ 90%), and photoperiod (18 light hours, keeping photosynthetically active radiation at the canopy level at 55 µmol m^{-2} s^{-1} using 5 W and 5000 K type LED luminaries). Rooted cuttings were transplanted into a 28 L geotextile pot filled with a homogeneous mixture of coconut peat, rice cisco, spent mushroom substrate, and sawdust. The resulting plants were grown in the greenhouse at an 18 h photoperiod provided, with supplementary light from 18:00 hours to midnight, using 30 W and 6000 K type LED luminaries; thermal variation was 11°C to 27°C, for twelve weeks.

Treatments and experimental design

A randomized complete block design was used, with five treatments and four replications. Treatments consisted of the applications of three chemical agents recognized for their effect on the induction of sexual reversion processes in female flowers. T1: silver thiosulfate (STS), T2: aminoetoxyvinylglycine (AVG), and T3: 1, methyl-cyclopropene (1-MCP). Further, two combinations of light photoperiod, named T4 (LP1 - 12-h light (hl) and 12-h dark (hd) photoperiod) and T5 (LP2 - 12hl:4hd:1hl:3hd:1hl:3hd photoperiod).

Sexual reversal

Aqueous solutions of STS and AVG were prepared in concentrations of 10 µg using Tween-80 (0.01%) as a surfactant. With a micropipette, a drop of 10 µL (solution) was applied to the apical meristem of the main shoot for five consecutive days at the end of the treatments, and each plant received 50 µg of each chemical compound. For the treatment with 1-MCP (gas activated on contact with water), the plants were hermetically isolated for 12 hours after application; a 2.5 g sachet with 14% of the active ingredient was used daily for five consecutive days. In LP2 treatment, the plants were isolated using a dark cover to conserve light at night. The control plants (LP1) only applied the solution of tween-80 (0.01%).

Light-saturation curve

The light saturation point at which the photosynthetic rate reaches its maximum was estimated using a portable photosynthesis system (LCi - ADC Bioscience, UK) provided by an external halogen lamp system (LCi - ADC Bioscience, UK), determining net photosynthetic (A, µmol CO_2 m^{-2} s^{-1}) to several photosynthetic photon flux density (PPFD: 220, 440, 660, 880, 1100, 1320, 1540, 1760, 1980 and 2200 µmol photons m^{-2} s^{-1}).

Gas-exchange and Chlorophyll fluorescence

Net photosynthetic rate (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) were determined using a portable photosynthesis system (LCi - ADC Bioscience, UK). An external halogen lamp (LCi - ADC Bioscience, UK) provided constant irradiation (1,320 µmol photons m^{-2} s^{-1}, PAR). Water use efficiency (*WUE*) was calculated using the A/E ratio and radiation use efficiency (*RUE*) by the *A*/*PAR* ratio. Measurements were made for 11 days (four days before, five days during, and two days after the treatment application) in one young adult leaf between 9:00 am and 11:00 am. Chlorophyll fluorescence was measured with a pulse modulated fluorometer (OS1p, Opti-Sciences, Hudson, NH). The maximum quantum efficiency of photosystem II – *Qy* [(Fv/Fm), non-photochemical quenching - *NPQ*, and rate of electron transport - *ETR* were estimated on one leaf previously dark acclimated for 30 min by using light exclusion clips (Lichtenthaler *et al.*, 2005).

Flower harvesting was carried out 20 days after suppressing additional light (12/12 h photoperiod) by collecting flowers in the last 10 cm of the central apical meristem. The number of male flowers was estimated by counting the number of flowers with the help of a stereoscope.

Statistical analysis

The models compared to describe a light-saturation curve were Logistic, Log-logistic, and Gompertz, which were adjusted to the photosynthesis of each PPFD using fixed and mixed-effects regression

models of 'drm' procedure in the library "drc" procedure in "R" software. Multiple model selection criteria were used: Bayesian information criteria (BIC), Akaike's information criterion (AIC), RMSE (root mean squared error), Mean absolute error (MAE) and R^2 (Willmott & Matsuura, 2005; Liu & Yang, 2011). The selected model was derived to validate the existence of possible critical points (maximum and minimum), which would allow identifying the light saturation point (LSP) of photosynthesis for *Cannabis*. One-way ANOVA analyzed the treatments and the mean multiple comparisons by Tukey's HSD (honestly significant difference) test (p<0.05). The Kruskal-Wallis test (p<0.05) was applied when the data did not meet the assumptions of ANOVA. All analyses were carried out using the R project "agricolae" package.

RESULTS

Light-saturation curve

Selection criteria (AIC, BIC, MAE, RMSD, and R^2) used to select the best model are shown in Table No. 1. For Logistic and Log-logistic model estimation, three, four, and five parameters were used, while the Gompertz model used three and four parameters. Although the best goodness-of-fit criteria (lower AIC, BIC, MAE, RMSD, and higher R^2) were observed to estimate all models when four parameters were used, not all four parameters showed significance. On the other hand, when three parameters were calculated for each model, a significance difference (Student T-test, p<0.001) was found in each of the three parameters evaluated for all models. The Log-logistic model was the one that achieved a better fit of the data; since it presented the lowest values for AIC, BIC, MAE, RMSD criteria, and the highest R^2 (Table No. 1). Log-Logistic equation-based best fit light saturation curve for net photosynthesis (A) by different levels of photosynthetic photon flux densities (PPFD) is shown as eq. [1]. The overall goodness of fit between the measured in A data and those fitted with the Log-Logistic equation for R^2 (0.44).

$$A = \frac{20.63657}{(1+\exp((-2.21229(\ln(x)-\ln(400.26552))))} \quad \text{eq. [1]}$$

Where A is Net photosynthesis, and x is photosynthetic photon flux density (*PPFD*).

The adjusted equation [1] for net photosynthesis in hemp plants showed a vertical asymptote (A = 20.63 µmol CO_2 m^{-2} s^{-1}) related to the maximum biological value, understood as the maximum rate of carbon fixation achieved by this species under this study conditions. On the other hand, regarding the light compensation point (LCP), that is, where A is equal to zero, to consider in equation [1] values of A close to 0.1 or 0.2 CO_2 m^{-2} s^{-1}, it was determined that this photosynthetic activity is achieved when the PPFD is 37 to 49 µmol photons m^{-2} s^{-1}, respectively.

The criterion of the first derivative was used to estimate the light saturation point of photosynthesis (LSP) in hemp plants and to find the specific maximum and minimum values. Figure No. 1 shows the first derivative of the Log-logistic model adjusted for A variable, where a positive trend is observed, indicating that an increase in PPFD provides an increase in A, with the rate of rapid variation being more significant for the first radiation levels (220 to 1,100 µmol of photons). From 1,100 µmol, the variation becomes uniform, with a tendency to zero, but without reaching this value, indicating the absence of a maximum value, that is, that from the criterion of the first derivative, it is not possible to determine the point of light saturation view of hemp under the evaluated conditions. Therefore, since A does not present a normal distribution according to the Shapiro-Wilk test (p<0.05), the Kruskal-Wallis test was performed to determine the point (PPFD) from which photosynthesis does not increase significantly (LSP) (Figure No. 1).

Kruskal-Wallis test classified photosynthetic rates in six groups (Figure No. 1). A value showed significant differences at radiation between 220 and 880 µmol of photons m^{-2} s^{-1}, with an increase of 86.11%. A second considerable increase (10.24%) was observed between 880 and 1,320 µmol of photons m^{-2} s^{-1}, while from 1,320 µmol of photons m^{-2} s^{-1}, nonsignificant differences in photosynthetic activity were observed. According to the above, hemp plants' light saturation point corresponded to a PPDF of 1,320 µmol photons m^{-2} s^{-1} since photosynthesis did not vary significantly from this radiation with the increase in PPFD (Figure No. 1).

Gas Exchange

Variables evaluated (A, E, gs, and WUE) with a significance level of p<0.05 are shown in Table No. 2. As observed, during the first four days before applying sexual reversal methods (fifth day), A values showed a significant difference between treatments; however, after the fifth day, A did not

show a significant difference from the fifth day to the eleventh day between treatments. Significant differences (p=0.00277) were observed on the twelfth day of evaluation, where LP1 was the treatment with the lowest A value (0.36 µmol CO_2 m^{-2} s^{-1}). Regarding E, hemp plants showed significant differences every day between treatments in the transpiration values. From the fifth day, E values mean in hemp plants LP1 (1.91 mmol H_2O m^{-2} s^{-1}) were significantly (p=0.0017) lower concerning 1-MCP (2.90 mmol H_2O m^{-2} s^{-1}) and STS (2.68 mmol H_2O m^{-2} s^{-1}) treatments. Referring to stomatal conductance (gs), only statistical differences were shown until the seventh day. Still, no effect of the treatments was observed in the following days, as reported for net photosynthesis (Table No. 2). On the other hand, water use efficiency (WUE) showed a significant difference between hemp plants treated with 1-MCP and AVG (p<0.05), being less efficient than the LP1 treatment. Likewise, in the WUE, LP1 presented significant differences, respect to STS (p=0.0341), LP2 (p=0.0025), and 1-MCP (p=0.0000), with mean values of 1.84 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} for respect to 1.457, 1.259, and 1.219 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} for STS, LP2 and 1-MCP respectively.

Table No. 1
Goodness-of-fit criteria for the studied regression for photosynthesis saturation light curve in Cannabis. Bayesian information criteria (BIC), Akaike's information criterion (AIC), RMSE (root mean squared error), and MAE (mean absolute error) for Logistic, Log-Logistic, and Gompertz models

Criteria	Logistic			Log-logistic			Gompertz	
	3*	4	5	3*	4	5	3*	4
AIC	3,324.7	3,318.6	3,320.7	3,316.4	3,318.0	3,320.0	3,319.7	3,318.6
BIC	3,341.8	3,340.1	3,346.4	3,333.6	3,339.5	3,345.8	3,336.9	3,340.0
MAE	3.39803	3.34716	3.3473	3.34637	3.34600	3.34613	3.36263	3.34702
RMSE	5.21790	5.17927	5.1795	5.17830	5.17627	5.17636	5.19396	5.17894
R^2	0.44181	0.46982	0.4697	0.46517	0.47033	0.47004	0.45821	0.46992

***Significant difference for the three parameters, student test (p<0.01)**

As a clear relationship between *A*, *E*, *gs*, and *WUE* in response to the sexual reversal treatments was not observed in hemp plants, the gas exchange data were grouped into two evaluation periods, moment one, before applying the treatments, and moment two, after application. For this, the value for each grouped variable was compared through Duncan multiple comparison test (p<0.05) (Table No. 3). Net photosynthesis in hemp plants decreased significantly (p=0.000144) after sexual reversal induction for LP1 (28%), 1-MCP (25%), STS (25%); however, in AVG (3.33 µmol CO_2 m^{-2} s^{-1}) and LP2 (3.71 µmol CO_2 m^{-2} s^{-1}), *A* values did not vary significantly (Table No. 3). *E* values showed a significant reduction (p=0.0042) between before and after periods for LP1 (28%) and STS (11%). Hemp plants treated with AVG (2.34 mmol H_2O m^{-2} s^{-1}), 1-MCP (2.89 mmol H_2O m^{-2} s^{-1}), and LP2 (3.01 mmol H_2O m^{-2} s^{-1}) did not show significant differences for *E*. Similarly; *gs* did show no significant differences for 1-MCP (0.075 mol CO_2 m^{-2} s^{-1}), AVG (0.06 mol m^{-2} s^{-1}), STS (0308 mol m^{-2} s^{-1}), and LP2 (0.07 mol m^{-2} s^{-1}) in both moments (before and after), contrary to observed in LP1, where *gs* decreased a 29% (p=0.0009) significantly.

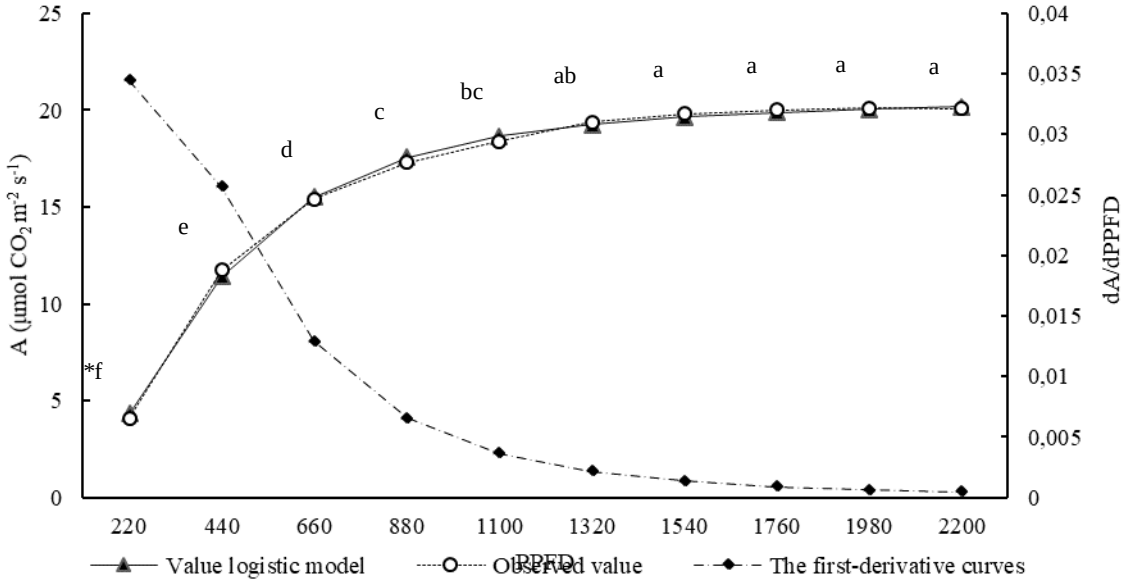

Figure No. 1
Net photosynthesis (*A*) of *Cannabis sativa* to different levels of photosynthetic photon flux densities (PPFD) and the first-derivate curve for Log-logistic model (3 parameters). *Different letters mean significantly different values (Kruskal-Wallis $p<0.05$)

Chlorophyll fluorescence

Values for the chlorophyll fluorescence parameter are shown in Table No. 4. For all variables evaluated, there were no significant differences. Quantum yield (Fv/Fm), electron transport rate (ETR), and non-photochemical quenching did not respond in a significant way to the application of chemical treatments or the alteration of photoperiod as a strategy to revert the sexuality of the female hemp plants (Table No. 4). Like gas exchange variables, chlorophyll fluorescence data were grouped into two evaluation periods before and after applying sexual reversal treatments. Each grouped variable evaluated was compared with Duncan's multiple comparison tests ($p<0.05$).

Significant differences were observed for Qy (Fv/Fm) ($p=0.0267$), ETR ($p=0.0317$), and NPQ ($p=0.000794$) about before and after periods of the applied treatments. Quantum yield PSII (Qy) and electron transport rate (ETR) increased after sexual reversal treatments by 1.32 and 9.08%, respectively; in contrast, non-photochemical quenching (NPQ) was significantly reduced by 28.15% (Table No. 5).

Flowering

Figure No. 2 illustrates the values obtained for the variable number of male flowers. All chemical treatments were effective in the sexual reversal of female hemp plants. However, there are significant differences ($p=0.00362$) for the number of male flowers; Nevertheless, LP1 and LP2 did not induce sexual reversal in female hemp plants. Using 1-MCP and AVG did not present a statistical difference between them, with an average production of 6.25 female flowers per plant. 1-MCP and AVG differed significantly from STS, which only allowed the reversion of 0.5 male flowers per plant.

Table No. 2
Gas Exchange: response of Cannabis plants treated with silver thiosulfate (STS), aminoetoxyvinylglycine (AVG), 1, methyl-cyclopropene (1-MCP), and two combinations of light photoperiod LP1: 12-h light (hl) and 12-h dark (hd) photoperiod and LP2: 12hl:4hd:1hl:3hd:1hl:3hd photoperiod

Variable	Treatment	Day before treatments				Treatments days					Days after treatment		
		1**	2	3	4**	5	6	7	8	9	10	11	12**
A	1MCP	5.26 a	4.08	2.05 bc	4.62 bc	2.86	2.34	3.92	2.90	3.05	2.92	3.98	1.97 ab
	AVG	2.89 c	2.94	2.56 ab	4.43 c	2.09	3.25	4.15	3.55	4.06	3.43	4.01	3.16 a
	STS	4.65 ab	4.21	2.73 abc	7.47 a	3.63	4.47	4.81	2.89	4.71	3.38	3.79	1.99 ab
	LP1	5.46 a	3.11	3.83 a	6.62 ab	2.62	2.74	4.69	4.70	3.52	3.53	5.56	0.36 b
	LP2	3.50 bc	3.65	2.23 c	5.90 abc	2.15	3.37	5.14	3.39	3.89	2.87	4.93)	3.12 a
		1**	2**	3*	4**	5**	6**	7*	8	9**	10*np	11**	12*
E	1MCP	3.04 a	3.02 ab	2.33 ab	3.19 b	2.05 a	2.86 ab	3.14 ab	2.63	3.59 ab	1.98 a	4.38 a	2.57 a
	AVG	2.11 b	2.2 bc	2.22 b	2.96 b	1.58 ab	1.95 b	2.93 b	2.40	2.89 bc	1.96 a	2.83 b	1.95 ab
	STS	2.64 ab	2.88 abc	3.14 a	4.20 a	1.66 ab	3.28 a	3.82 a	2.31	3.28 bc	1.67 ab	3.41 b	2.08 ab
	LP1	2.33 ab	1.88 c	2.91 ab	3.45 ab	1.02 b	1.89 b	2.75 b	2.21	2.25 c	1.44 b	2.98 b	0.78 b
	LP2	2.83 a	3.12 a	2.59 ab	4.22 a	1.38 ab	2.34 ab	3.72 ab	2.59	4.26 a	2.05 a	4.89 a	2.76 a
		1*np	2*np	3*np	4*	5*	6*	7	8	9	10	11*np	12
gs	1MCP	0.088 a	0.070 ab	0.045 b	0.078 b	0.084 a	0.070 ab	0.070	0.091	0.073	0.088	0.097 ab	0.041
	AVG	0.053 b	0.050 b	0.048 ab	0.073 ab	0.060 ab	0.050 b	0.068	0.083	0.060	0.088	0.064 c	0.037
	STS	0.080 a	0.080 a	0.063 a	0.117 a	0.068 ab	0.091 a	0.094	0.090	0.080	0.078	0.085 abc	0.036
	LP1	0.065 ab	0.055 ab	0.055 ab	0.105 ab	0.037 b	0.042 ab	0.057	0.080	0.052	0.060	0.070 bc	0.012
	LP2	0.070 a	0.072 ab	0.056 b	0.092 ab	0.053 b	0.056 ab	0.083	0.084	0.081	0.088	0.108 a	0.045
		1*	2**	3	4*	5**	6	7	8**	9**	10**	11**	12**
WUE	1MCP	1.74 a	1.41 b	0.87	1.55 ab	1.47 c	0.97	1.32	1.16 b	0.90 b	1.58 bc	0.93 b	0.73 bc
	AVG	1.39 b	1.32 b	1.25	1.5 b	1.38 c	1.54	1.48	1.42 b	1.34 ab	1.83 abc	1.26 ab	1.65 a
	STS	1.77 a	1.46 b	0.87	1.81 ab	2.15 ab	1.32	1.23	1.22 b	1.36 ab	2.11 ab	1.11 b	1.09 abc
	LP1	2.34 a	2.00 a	1.38	2.0 a	2.52 a	1.36	1.76	2.18 a	1.59 a	2.53 a	1.78 a	0.73 c
	LP2	1.26 b	1.20 b	0.76	1.39 b	1.73 bc	1.58	1.37	1.28 b	0.9 b	1.39 c	0.98 b	1.27 ab

***A*, net photosynthesis (umol m^{-2} s^{-1}); *E*, transpiration rates (mmol m^{-2} s^{-1}); *gs*, stomatal conductance (mol m^{-2} s^{-1}); WUE, water use efficiency (umol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}), Values are means (n=4). * Different letters in the same day mean significantly different values (Tukey test *p<0.05; **p<0.01). *np: Different letters in the same day mean significantly different values (Kruskal-Wallis, p<0.05)**.

Table No. 3
Net photosynthesis (*A*), transpiration rate (*E*), and stomatal conductance (*gs*) of hemp plants at different photosynthetic photon flux density (PPFD), after and before the treatment with different sexual reversal methods

	A **(µmol CO_2 m^{-2} s^{-1})**		***E*** **(mmol H_2O m^{-2} s^{-1})**		***gs*** **(mol CO_2 m^{-2} s^{-1})**	
	Before	**After**	**Before**	**After**	**Before**	**After**
1MCP	4 ± 1.69 ab	2.99 ± 1.23 c	2.89 ± 0.83 ab	2.9 ± 1.07 ab	0.07 ± 0.03 ab	0.08 ± 0.03 ab
AVG	3.2 ± 1.17 bc	3.46 ± 1.94 bc	2.37 ± 0.57 c	2.31 ± 0.93 cd	0.06 ± 0.02 c	0.06 ± 0.04 cd
STS	4.76 ± 2.54 a	3.71 ± 2.36 bc	3.21 ± 0.88 a	2.69 ± 1.15 bc	0.08 ± 0.04 ab	0.08 ± 0.04 bc
LP1	4.76 ± 1.94 a	3.53 ± 2.19 bc	2.64 ± 1 bc	1.94 ± 0.82 d	0.07 ± 0.04 bc	0.05 ± 0.02 d
LP2	3.82 ± 1.86) bc	3.61 ± 1.6 bc	3.19 ± 1 a	3 ± 1.39 ab	0.07 ± 0.03 a	0.07 ± 0.03 ab

*** Different letters mean significantly different values (Duncan p<0.05)**

Table No. 4
Chlorophyll fluorescence parameters of *Cannabis* plants treated with silver thiosulfate (STS), aminoetoxyvinylglycine (AVG), 1, methyl-cyclopropene (1-MCP), and two combinations of light photoperiod LP1: 12-h light (hl) and 12-h dark (hd) photoperiod and LP2: 12hl:4hd:1hl:3hd:1hl:3hd photoperiod

Parameters	Treatment	Day before treatments				Treatments days					Days after treatment		
		1	2	3	4	5	6	7	8	9	10	11	12
FV/Fm	1MCP	0.733	0.726	0.738	0.774	0.790	0.765	0.742	0.767	0.718	0.760	0.746	0.740
	AVG	0.737	0.729	0.733	0.759	0.772	0.759	0.761	0.777	0.730	0.766	0.726	0.738
	STS	0.731	0.718	0.712	0.759	0.773	0.768	0.721	0.754	0.727	0.767	0.736	0.749
	LP1	0.728	0.737	0.725	0.778	0.772	0.721	0.724	0.747	0.691	0.754	0.708	0.677
	LP2	0.747	0.732	0.726	0.766	0.770	0.749	0.733	0.756	0.744	0.756	0.753	0.752
ETR	1MCP	1.170	1.170	1.25	0.900	0.775	1.375	1.375	1.250	1.275	1.450	1.475	1.075
	AVG	1.200	1.325	1.275	0.75	0.780	1.300	1.250	1.275	1.35	1.275	1.300	1.450
	STS	1.070	1.175	1.100	0.725	0.575	1.375	1.200	1.075	1.275	1.275	1.125	1.050
	LP1	1.200	1.400	1.100	0.750	0.450	1.100	1.250	1.050	1.310	1.050	1.300	0.950
	LP2	1.170	1.225	1.15	0.775	0.775	1.175	1.200	1.225	1.300	1.525	1.425	1.450
NPQ	1MCP	1.963	2.020	1.888	1.305	0.988 ab	1.378	1.668	1.433	1.913	1.388	1.698	1.655
	AVG	1.730	1.923	1.903	1.290	0.823 ab	1.475	1.670	1.405	1.708	1.335	1.630	1.750
	STS	1.825	1.965	1.263	1.118	0.798 b	1.253	1.658	1.188	1.733	1.205	1.58	1.783
	LP1	1.745	1.660	1.580	1.015	0.855 ab	1.515	1.890	1.230	1.860	1.385	1.145	1.83
	LP2	1.828	1.953	1.908	1.205	1.188 a	1.740	1.755	1.538	1.873	1.443	1.670	1.808

Values are means of Fv/Fm, PSII maximum quantum yield; ETR, electron transport rate; and NPQ, non-photochemical quenching

Table No. 5
Chlorophyll fluorescence parameters of hemp plants after and before the treatment with different sexual reversal methods

Moment	Fv/Fm	ETR	NPQ
Before	0.74 ± 0.03 b	1.09 ± 0.3 b*	1.64 ± 0.51 a
After	0.75 ± 0.03 a	1.2 ± 0.37 a	1.5 ± 0.38 b

Fv/Fm, PSII maximum quantum yield (Qy); ETR, electron transport rate and NPQ, non-photochemical quenching coefficient. Values are means (± S.E.) for three plants. *Different letters in the same column mean significantly different values Duncan test $p<0.05$).

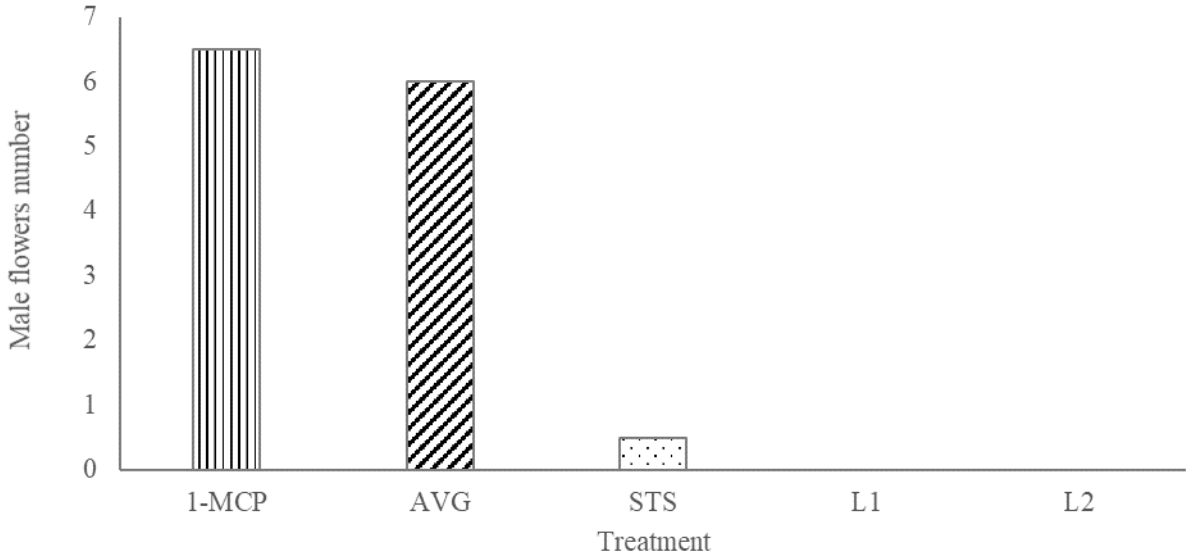

Figure No. 2
Males flowers number of Cannabis plants treated with silver thiosulfate (STS), aminoetoxyvinylglycine (AVG), 1, methyl-cyclopropene (1-MCP), and two combinations of light photoperiod LP1: 12-h light (hl) and 12-h dark (hd) photoperiod and LP2: 12hl:4hd:1hl:3hd:1hl:3hd photoperiod. Values are means (± S.E.) for three plants. *Different letters in the same day mean significantly different values (Duncan $p<0.05$)

DISCUSSION

The ability of hemp plants to keep the photosynthetic rate active even at high radiation levels made it difficult to determine a theoretical light saturation point. Since *A* values increased as a function of PPDF, it is indicated that hemp is a species adapted to high radiation conditions. However, hemp plants can reach a considerable photosynthetic rate even in medium radiation (Figure No. 1). The above agrees with Chandra *et al*. (2008), who indicated that photosynthesis in hemp plants is highly influenced by the intensity and quality of the plants' light. Our results are like those obtained by Chandra *et al*. (2015) and Tang *et al*. (2017), where the maximum values of *A* in hemp plants were observed between 1,300 and 1,500 µmol of photons m^{-2} s^{-1} at a temperature of 30°C, while lower PPDF reduced CO_2 assimilation capacity and therefore yield, while higher values, did not have a significant effect on this capacity. Differences between *A* values shown in Figure No. 1 and Table No. 2 concerning the variations in the assimilation of CO_2 can be explained as reported by Bauerle *et al*. (2020), who found a considerable decrease in *A* capacity of hemp leaf with respect to age, where the highest rate was achieved in leaves aged up to five days (20 to 25 µmol CO_2 m^{-2} s^{-1}), with a reduction significant at values between 4 to 10 µmol CO_2 m^{-2} s^{-1} in leaves older than 15 days of development. In our experiment, the light saturation curve was performed on fully developed young leaves with maximum values of *A* (19.37 ± 0.77 µmol CO_2 m^{-2} s^{-1}). This same leaf was used for the gas exchange measurements carried out after 13 days of full leaf expansion, showing similar results in this variable to those reported by Bauerle *et al*. (2020).

Regarding light compensation point (LCP) for hemp plants, this parameter is unknown, a LCP of 37 to 49 µmol photons m^{-2} s^{-1} is considered low, similar to that reported in other species C_3 as cabbage by Lefsrud *et al*. (2019) who determined LCP from 13 to 23 µmol photons m^{-2} s^{-1}. According to Sterck *et al*. (2013), plants with a lower LCP can tolerate deeper shade than plants with a higher LCP; in this sense, it suggested that plants that gain greater carbon under shaded conditions increase the plant shade tolerance. LSP and LCP determination is important because more and more cannabis producers are moving to closed growth chambers where light quality, light intensity, and photoperiod play an essential role in a successful growth protocol (Magagnini *et al*., 2018). Although Cannabis is a plant adapted to high irradiance levels, there is no

evidence that a higher photosynthesis rate equals a higher flower yield. It is also questionable whether such a high light intensity (1,500 PPFD) is economically viable in terms of energy costs put, or what is the least economically viable radiation since greenhouse or indoor production has been classified as one of the energy-intensive industries (Chandler, 2011; Magagnini *et al*., 2018).

Photosynthesis and chlorophyll fluorescence parameters were used in the present study to evaluate the physiological responses of hemp plants to sexual reversal treatments. It has been shown that phytohormones play a fundamental role among the various factors that regulate gas exchange (Khan, 2006). This result explains that photosynthetic activity in hemp plants was reduced after sexual reversal treatments (Table No. 2). Various studies report this regulatory effect, like Shivashankara and Mathai (2000) and Urban *et al*. (2004), who found in *Mangifera indica* higher *A* and *gs* values in leaves of branches without flowers, regarding *A* values in branches in the flowering stage. This effect is attributed to the reduction in the decarboxylation efficiency of Rubisco, associated with possible inhibitors such as abscisic acid (ABA) present in the leaves of the flowering branches. On the other hand, Pallas & Kays (1982) attribute the decrease in photosynthetic activity in peanut plants (*Arachis hypogaea*) to ethylene's role in carbon fixation, increasing internal CO_2 concentrations, which promotes stomatal closure.

AVG and 1-MCP were effective in the sexual reversion of female hemp plants and presented differences in *A*. On the other hand, hemp plants treated with AVG maintained stable *A* values, which is consistent with what was found by Najeeb *et al*. (2015), who highlight the role of AVG in the regulation of photosynthetic activity in cotton plants. On the other hand, 1-MCP did not present differences for this variable to the control plants, which is similar to what was found by Loka & Oosterhuis, (2013), which report that 1-MCP cannot prevent a photosynthetic decrease in cotton plants.

It has been documented that ethylene, a key regulator of plant growth and development, acts as a signaling molecule, whit a significant role in plant responses to external stimuli. Ethylene improves the adaptation and productivity of agricultural systems by the regulation of photosynthesis and several processes, including seed germination, sex determination, leaf, stem, and root growth, fruit ripening, abscission of plant organs, and senescence (Zhang *et al*., 2014; Iqbal *et al*., 2017; Khan *et al*., 2020). Under unfavorable conditions, high ethylene production has been attributed to the changes in photosynthesis due to its effects on stomatal conductance (Khan, 2006). Consequently, water's reduced evapotranspiration and reactive oxygen species (ROS) accumulation are observed (Riyazuddin *et al*., 2020). Therefore, applying ethylene inhibitors such as 1-MCP, AVG, or STS reduces the ethylene and its precursors aminocyclopropane-carboxylic (ACC) concentration (Zhang *et al*., 2009; Hussain *et al*., 2018)

In the present study, applying the ethylene inhibitor (1-MCP, AVG, STS) reduced net photosynthesis in hemp plants after the LP1, 1-MCP, and STS treatments. The belove mentioned differs from reported by Zhang *et al*. (2014) and Li *et al*. (2012) in rice, where the reduction of the action of ethylene has effects on the improvement of the net photosynthetic, the dry matter of the plant, and the partition of the assimilated to the panicle. According to Khan *et al*. (2020), this is due to negative regulation of the response to ABA, leading to the conservation of carbohydrates, which promotes a photosynthetic rate reduction.

On the other hand, in terms of transpiration rate and stomatic conductance, these variables were not affected by ethylene inhibitors, which remained constant after applying sexual reversal treatments. Tholen *et al*. (2008) indicate that the negative effect of ethylene for the inhibition of photosynthesis is on stomatal conductance. However, Khan (2004), argues that ethylene may stimulate photosynthesis, regardless of its effect on stomatal conductance. The decrease in photosynthetic activity and stomatal conductance observed in control plants suggests possible participation of ABA in reducing stomatal conductance, therefore, a CO_2 reduction. In contrast, plants treated with AVG and 1-MCP maintained constant *gs* values, suggesting that they generate indirect control over the action of ABA by regulating ethylene. However, the fact that AVG keeps photosynthetic rates in contrast to 1-MCP indicates the participation of some other type of compound in the regulatory process of photosynthesis.

None of the chlorophyll fluorescence parameters varied due to the treatments applied for sexual reversal. It can be inferred that these variables didn't work like indicators of physiological changes derived from the processes of natural floral differentiation or modified by chemical agents such as 1-MCP, AVG or STS, or discontinuous alterations

in the light regime. However, floral induction as a response to alterations in the photoperiod of *Cannabis* plants showed considerable increases in ETR. Moradi & Ismail, (2007) found similar results, who report an increase in the ETR in rice plants in the reproductive stage concerning the vegetative one. Qy and ETR increase and a NPQ decrease are presumed to indicate an optimization in the way plants transfer the energy captured by light-harvesting complexes in Q_A oxide reduction. As photoassimilates for tissue formation, this energy transference in the different metabolic pathways can be considered an indicator of flowering and secondary metabolites (cannabinoids) production.

CONCLUSIONS

Gas exchange parameters are a general indicator of sexual reversal in hemp plants concerning applying all exogenous chemicals. Induction of sexual reversion in hemp plants does not change the mechanisms as plants dissipate energy through photosystems, suggesting that chlorophyll fluorescence is not an indicator for this physiological process. Although 1-MCP has not been reported as a sexual reversal for female plants, it showed great potential for this use in *Cannabis* plants; therefore, research should be expanded for this purpose.

REFERENCES

Ablin J, Ste-Marie PA, Schäfer M, Häuser W, Fitzcharles MA. 2016. Medical use of cannabis products. **Der Schmerz** 30: 3 - 13. **https://doi.org/10.1007/s00482-015-0083-4**

Bauerle WL, McCullough C, Iversen M, Hazlett M. 2020. Leaf age and position effects on quantum yield and photosynthetic capacity in hemp crowns. **Plants** 9: 271. **https://doi.org/10.3390/plants9020271**

Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, Mastinu A. 2018. *Cannabis sativa:* A comprehensive ethnopharmacological review of a medicinal plant with a long history. **J Ethnopharmacol** 227: 300 - 315. **https://doi.org/10.1016/j.jep.2018.09.004**

Chailakhyan MK, Khryanin VN. 1978. The influence of growth regulators absorbed by the root on sex expression in hemp plants. **Planta** 138: 181 - 184.

Chandler JW. 2011. The hormonal regulation of the flower development. **J Plant Growth Regul** 30: 242 - 254. **https://doi.org/10.1007/s00344-010-9180-x**

Chandra S, Lata H, Khan IA, Elsohly MA. 2008. Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO_2 conditions. **Physiol Mol Biol Plants** 14: 299 - 306. **https://doi.org/10.1007/s12298-008-0027-x**

Chandra S, Lata H, Mehmedic Z, Khan IA, ElSohly MA. 2015. Light dependence of photosynthesis and water vapor exchange characteristics in different high Δ^9-THC yielding varieties of *Cannabis sativa* L. **J Appl Res Med Aromat Plant** 2: 39 - 47. **https://doi.org/10.1016/j.jarmap.2015.03.002**

Cho LH, Pasriga R, Yoon J, Jeon JS, An G. 2018. Roles of sugar in controlling flowering time. **J Plant Biol** 61: 121 - 130. **https://doi.org/10.1007/s12374-018-0081-z**

Clarke RC, Merlin MD. 2013. ***Cannabis* evolution and ethnobotany**. University of California Press Los Angeles, California, USA.

Duca M. 2015. Photosynthesis. **Plant Physiol** 65-122. Springer International Publishing. Germany.

Ebbert JO, Scharf EL, Hurt RT. 2018. Medical cannabis. **Mayo Clin Proc** 93: 1842 - 1847. **https://doi.org/10.1016/j.mayocp.2018.09.005**

Grotenhermen F. 2006. Los cannabinoides y el sistema endocannabinoide. **Cannabinoids** 1: 10 - 14.

Hall J, Bhattarai SP, Midmore DJ. 2012. Review of flowering control in industrial hemp. **J Nat Fibers** 9: 23 - 36. **https://doi.org/10.1080/15440478.2012.651848**

Heslop-Harrison J. 2018. **Objectives and organization of the work**. In: Kinet JM, Sachs RM, Bernier G (eds.). The physiology of flowering. Volume III. The development of flowers. CRC Press, Boca Raton, Florida, USA.

Hobza R, Hudzieczek V, Kubat Z, Cegan R, Vyskot B, Kejnovsky E, Janousek B. 2018. Sex and the flower–developmental aspects of sex chromosome evolution. **Ann Bot** 122: 1085 - 1101. **https://doi.org/19.1093/aob/mcy130**

Hussain S, Zhong C, Bai Z, Cao X, Zhu L, Hussain A, Zhu C, Fahad S, James AB, Zhang J, Jin Q. 2018. Effects of 1-Methylcyclopropene on rice growth characteristics and superior and inferior spikelet development under salt stress. **J Plant Growth Regul** 37: 1368 - 1384. **https://doi.org/10.1007/s00344-018-9800-4**

Iqbal N, Umar S, Per TS, Khan NA. 2017. Ethephon increases photosynthetic-nitrogen use efficiency, proline and antioxidant metabolism to alleviate decrease in photosynthesis under salinity stress in mustard. **Plant Sign Behav** 12: e1297000. **https://doi.org/10.1080/15592324.2017.1297000**

Khan NA. 2004. An evaluation of the effects of exogenous ethephon, an ethylene releasing compound, on photosynthesis of mustard *Brassica juncea* cultivars that differ in photosynthetic capacity. **BMC Plant Biol** 4. **https://doi.org/10.1186/1471-2229-4-21**

Khan NA. 2006. **Ethylene involvement in photosynthesis and growth**. In ethylene action in plants. Springer, Heidelberg, Berlin, Germany. **https://doi.org/10.1007/978-3-540-32846-9_9**

Khan MIR, Trivellini A, Chhillar H, Chopra P, Ferrante A, Khan NA, Ismail AM. 2020. The significance and functions of ethylene in flooding stress tolerance in plants. **Envirol Exp Bot** 179: 104188. **https://doi.org/10.1016/j.envexpbot.2020.104188**

Lefsrud M, Eichhorn BS, Wu BS, Rufyikiri AS, MacPherson S. 2019. An update on plant photobiology and implications for *Cannabis* production. **Front Plant Sci** 10: 15. **https://doi.org/10.3389/fpls.2019.00296**

Li G, Meng X, Wang R, Mao G, Han L, Liu Y. 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in Arabidopsis. **Plos Genet** 8: e1002767. **https://doi.org/10.1371/journal.pgen.1002767**

Lichtenthaler HK, Buschmann C, Knapp M. 2005. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. **Photosynthetica** 43: 379 - 393.

Liu W, Yang Y. 2011. Parametric or nonparametric? A parametricness index for model selection. **Ann Statist** 39: 2074 - 2102. **https://doi.org/10.1214/11-AOS899**

Loka DA, Oosterhuis DM. 2013. Effect of 1-MCP on gas exchange and carbohydrate concentrations of the cotton flower and subtending leaf under water-deficit stress. **Am J Plant Sci** 4: 142 - 152. **https://doi.org/10.4236/ajps.2013.41019**

Lubell JD, Brand MH. 2018. Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. **HortTechnology** 28: 743 - 747. **https://doi.org/10.21273/HORTTECH04188-18**

Magagnini G, Grassi G, Kotiranta S. 2018. The effect of light spectrum on the morphology and cannabinoid content of *Cannabis sativa* L. **Med Cannabis Cannabinoids** 1: 19 - 27. **https://doi.org/10.1159/000489030**

Mansouri H, Salari F, Asrar Z. 2013. Ethephon application stimulats cannabinoids and plastidic terpenoids production in *Cannabis sativa* at flowering stage. **Ind Crops Prod** 46: 269 - 273. **https://doi.org/10.1016/j.indcrop.2013.01.025**

Moliterni VMC, Cattivelli L, Ranalli P, Mandolino G. 2004. The sexual differentiation of *Cannabis sativa* L.: A morphological and molecular study. **Euphytica** 140: 95 - 106.

Moradi F, Ismail AM. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. **Ann Bot** 99: 1161 - 1173. **https://doi.org/10.1093/aob/mcm052**

Najeeb U, Atwell BJ, Bange MP, Tan DKY. 2015. Aminoethoxyvinylglycine (AVG) ameliorates waterlogging-induced damage in cotton by inhibiting ethylene synthesis and sustaining photosynthetic capacity. **Plant Growth Regul** 76: 83 - 98. **https://doi.org/10.1007/s10725-015-0037-y**

Pallas JE, Kays SJ. 1982. Inhibition of photosynthesis by ethylene-a stomatal effect. **Plant Physiol** 70: 598 - 601. **https://doi.org/10.1104/pp.70.2.598**

Ram HM, Sett R. 1982a. Modification of growth and sex expression in *Cannabis sativa* by aminoethoxyvinylglycine and ethephon. **J Plant Physiol** 105: 165 - 172.

Ram HM, Sett R. 1982b. Reversal of ethephon-induced feminization in male plants of *Cannabis sativa* by ethylene antagonists. **J Plant Physiol** 107: 85 - 89.

Ram HM, Sett R. 1982c. Induction of fertile male flowers in genetically female *Cannabis sativa* plants by silver nitrate and silver thiosulphate anionic complex. **Theor Appl Genet** 62: 369 - 375.

Razumova OV, Alexandrov OS, Divashuk MG, Sukhorada T, Karlov G. 2015. Molecular cytogenetic analysis of monoecious hemp (*Cannabis sativa* L.) cultivars reveals its karyotype variations and sex chromosomes constitution. **Protoplasma** 253: 895 - 901. **https://doi.org/10.1007/s00709-015-0851-0**

Riyazuddin R, Verma R, Singh K, Nisha N, Keisham M, Bhati KK, Kim ST, Gupta R. 2020. Ethylene: A master regulator of salinity stress tolerance in plants. **Biomolecules** 10: 959.

https://doi.org/10.3390/biom10060959

Samuolienė G, Duchovskis P. 2012. **Interaction between flowering initiation and photosynthesis**. In: (ed.). Najafpour M.M. Applied photosynthesis. InTech. Croacia.

Shivashankara KS, Mathai CK. 2000. Inhibition of photosynthesis by flowering in mango (*Mangifera indica* L.). A study by gas exchange methods. **Sci Hortic** 83: 205 - 212. **https://doi.org/10.1016/S0304-4238(99)00085-0**

Sterck FJ, Duursma RA, Pearcy RW, Valladares F, Cieslak M, Weemstra M. 2013. Plasticity influencing the light compensation point offsets the specialization for light niches across shrub species in a tropical forest understorey. **J Ecol** 101: 971 - 980. **https://doi.org/10.1111/1365-2745.12076**

Tang K, Struik P, Amaducci S, Stomph T, Yin X. 2017. Hemp (*Cannabis sativa* L.) leaf photosynthesis in relation to nitrogen content and temperature: implications for hemp as a bio-economically sustainable crop. **Glob Change Biol Bioenergy** 9: 1573 - 1587. **https://doi.org/10.1111/gcbb.12451**

Teskey RO, Sheriff DW, Hollinger DY, Thomas RB. 1995. External and internal factors regulating photosynthesis. In **Resource Physiology of Conifers: Acquisition, Allocation, and Utilization** 105 - 140. **https://doi.org/10.1016/B978-0-08-092591-2.50009-1**

Tholen D, Pons TL, Voesenek LA, Poorter H. 2008. The role of ethylene perception in the control of photosynthesis. **Plant Sign Behav** 3: 108 - 109. **https://doi.org/10.4161/psb.3.2.4968**

Truta E, Gille E, Tóth E, Maniu M. 2002. Biochemical differences in *Cannabis sativa* L. depending on sexual phenotype. **J Appl Genet** 43: 451 - 462.

Urban L, Lu P, Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. **Tree Physiol** 24: 387 - 399. **https://doi.org/10.1093/treephys/24.4.387**

Willmott CJ, Matsuura K. 2005. Advantages of the mean absolute error (MAE) over the root mean square error (RMSE) in assessing average model performance. **Clim Res** 30: 79 - 82. **https://doi.org/10.3354/cr030079**

Zhang H, Tan GL, Wang ZQ. 2009. Ethylene and ACC levels in developing grains are related to the poor appearance and milling quality of rice. **Plant Growth Regul** 58: 85 - 96. **https://doi.org/10.1007/s10725-008-9354-8**

Zhang JH, Zhu LF, Yu SM, Jin QY. 2014. Involvement of 1-methylcyclopropene in plant growth, ethylene production, and synthase activity of inferior spikelets in hybrid rice differing in panicle architectures. **J Plant Growth Regul** 33: 551 - 561. **https://doi.org/10.1007/s00344-013-9404-y**