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Distribution of Photosynthetic Pigments and Nitrate Nitrogen in the Leaves of Non-Traditional Fruit Crops as an Indicator of the Physiological State of Plants under the Conditions of Joint Cultivation

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The peculiarities of the accumulation of photosynthetic pigments and nitrate nitrogen in the leaves of *Actinidia arguta* (Sieboldet. Zucc.) Planch. ex Miq., *Schisandra chinensis* (Tucrz.) Bail., *Malus domestica* Borkh., *Cornus mas* L., *Chaenomeles japonica* (Thunb.) Lindl. ex Spach when grown in vegetation experiments with different ratios of the number of plants in containers, namely 50 : 50%, 33 : 67%, and 67 : 33%, were investigated. One-species plantings were used as a control. It was shown that the ratio between plants when grown together, affects the content of photosynthetic pigments and nitrate nitrogen in the leaves. Mixed plantings can reduce the concentration of photosynthetic pigments in the leaves of *A. arguta*. In mixed plantings, suppression of photosynthetic pigments in *A. arguta* leaves is observed, except for the variant with *C. japonica*. At the same time, a positive effect of actinidia was established for all other research cultures. When determining the compatibility of plants, the most indicative information is the ratio of the amount of chlorophylls to carotenoids. The opposite pattern was revealed between the content of photosynthetic pigments and nitrate nitrogen in the leaves of experimental crops. According to modern ideas, the biosynthesis of photosynthetic pigments should increase with a decrease in the concentration of nitrate nitrogen in the leaves, which was confirmed in our experiments. These results can be used in further allelopathic, physiological, and ecological investigations of different fruit plants.

Keywords: *Actinidia arguta*, *Schisandra chinensis*, *Malus domestica*, *Cornus mas*, *Chaenomeles japonica*, mixed plantings, photosynthetic pigments, nitrate nitrogen

Introduction

The qualitative indicators of the photosynthetic apparatus are evaluated by the composition of pigments in chloroplasts since spectrally different forms of pigments are involved in the process of photosynthesis in different ways and are unevenly distributed among individual photosystems. The study of the pigment complex in plants allows to reveal the degree of change and resistance of the photosynthetic apparatus to certain factors that activate its work. This approach makes it possible to reveal the dependence between the anatomical and physiological indicators of the photosynthetic apparatus and its activity, as well as to predict its reactions to the influence of exo- and endogenous factors for the development

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of physiological bases for the optimization of the photosynthetic apparatus, which can be carried out under controlled conditions (Colzodillo et al., 2022).

In general, photosynthetic productivity is enhanced by light, energy conversion, and carbon fixation (Hitchcock et al., 2022). At the same time, rapid adaptation to light leads to the accumulation of dry matter (Kromdijk et al., 2016; Hubbart et al., 2018). In addition, carbon fixation is enhanced as a result of the increased activity of enzymes of the Calvin-Benson cycle (Driever et al., 2017; Simkin et al., 2017; Mitchell et al., 2020).

It is known that various environmental factors, in particular drought, and high and low temperatures, negatively affect the processes of photosynthesis (Fahas et al., 2017). Biochemical composition and structural characteristics of leaves can affect their photosynthetic capacity (Wright et al., 2007). Two key traits that determine photosynthetic capacity are specific leaf area and leaf nitrogen content (Hikosaka, 2004; Poorter et al., 2009). Nitrogen is the main component of chlorophyll, as 70% of its content is accumulated in chloroplasts (Fathi and Zeidali, 2021; Moeinirad et al., 2021).

It should be noted that research in recent years has proven the fact that the biosynthesis of photosynthetic pigments is inhibited by the use of nitrate forms of nitrogen (Wang et al., 2022). Information regarding the elucidation of the role of NO in the processes of photosynthesis is limited, so understanding the function of nitrate nitrogen in the biosynthesis of photosynthetic pigments is important for optimizing plant growth and development.

Photosynthesis is the central metabolic process in plants, which combines physiological reactions, environmental factors, and biochemical changes in the cell, therefore the assessment of the variability of the structural and functional indicators of the photosynthetic apparatus, which characterize the adaptation of different species to the conditions of simultaneous growth, has important theoretical and practical significance since the issue of greening horticulture is extremely relevant. There is still a lack of data on the variability of the content of photosynthetic pigments in plants of different species, united by a commonplace of growth in a limited space. Therefore, the purpose of our work was to carry out research related to the peculiarities of the biosynthesis of photosynthetic pigments and the accumulation of nitrate nitrogen in the leaves of non-traditional fruit crops under conditions of mixed plantings.

Material and methodology

Plant material

The experiments were carried out in the Department of Acclimatization of Fruit Plants of the M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv). 2-year-old plants of *Actinidia arguta* (Sieboldet. Zucc.) Planch. ex Miq., *Schisandra chinensis* (Tucrz.) Bail., *Malus domestica* Borkh., *Cornus mas* L., *Chaenomeles japonica* (Thunb.) Lindl. ex Spach seedlings were chosen as objects of study. Plants were planted in March 2021 in containers with dark gray forest light loamy soil (humus content of the humate-fulvate type is 4.25%, pHKCl 7.6, nitrogen 2%, phosphorus 8.2%, density 1.2–1.4 g·cm^{-3}). The experiments were carried out under controlled conditions. The temperature was maintained within 22 ±2 °C, and soil humidity 60 ±5%. In the model experiment, for maximum saturation of the soil with root secretions, two-year-old plants were planted in containers of 12 pieces, with a distance of 5 cm between them. The surface area of the container was 0.06 m^2. The duration of the experiment was 24 weeks due to the generally accepted technology for growing experimental plant species.

Biochemical analysis

Pigment determination

Samples for biochemical studies were taken in mid-August. The content of photosynthetic pigments in the leaves was assessed using spectrometry SF-26. The extracts were prepared in a 96% alcohol solution for measurements. Carotenoids were determined at 440.5 nm, chlorophyll *a* at – 665 nm, and chlorophyll *b* – at 649 nm with 10 repetitions. The concentration of chlorophylls was calculated according to the Vermont formula, and carotenoids according to the Wettstein formula (Lichtenthaler and Wellburn, 1983):

$$\text{chlorophyll } a = 13.95\ A_{665} - 6.88\ A_{649}$$

$$\text{chlorophyll } b = 24.96\ A_{649} - 7.32\ A_{665}$$

$$C_{x+c} = (1000\ A_{470} - 2.05C_a - 114.8C_b)/245$$

The content of pigments in the extract was calculated by the following formula:

$$A = V \times C \Big/ (P \times 1{,}000)$$

where: C – the concentration of pigments, mg·l^{-1}; V – the volume of the extract, ml; P – the weight of plant material, g; A – pigment content in plant material, mg·kg^{-1} fresh weight (FW)

The obtained indicators, determined with a 95% confidence interval, are trustworthy due to the high reliability of the arithmetic mean values (the calculated Student‘s criterion significantly exceeds the tabular values) and the experimental error indicator is less than 5% using the ANOVA program.

Nitrate nitrogen determinations

The nitrate content of the samples was determined using a chemiluminescent detector. Briefly, an aliquot (5 µl) of vegetable juice was introduced into the reaction vessel of a chemiluminescent detector of a nitric oxide analyzer (Sievers NOA280i). This instrument directly detects NO and can be used to analyze nitrite and nitrate under conditions where these species are converted to NO. For nitrite analysis, the reaction vessel contained 1% w/v KI in glacial acetic acid to reduce nitrite to NO. For nitrate analysis, the reaction vessel contained a solution of vanadium (III) chloride in hydrochloric acid (1 M HCl) at 90 °C to reduce nitrite and nitrate to NO. Thus, the nitrate concentration is obtained by subtracting the measured nitrite concentration from the nitrite and nitrate concentration. Nitrite and nitrate concentrations were determined based on the standard curve (Huang et al., 2002).

Statistical analysis

All analyses were carried out in triplicate. The significance of differences between the OMP levels (significance level, p <0.05) was examined using the Kruskal-Wallis one-way analysis of variance. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 Software (TIBCO Software Inc., USA) (Zar, 1999).

Results and discussion

At the first stage of the research, a comparative analysis of the distribution of photosynthetic pigments in the leaves of *A. arguta*, *Sch. chinensis*, *M. domestica*, *C. mas*, and *Ch. japonica* plants was carried out under joint cultivation. It has been experimentally proven that under the conditions of mixed plantings of *A. arguta* and *M. domestica*, there is an inhibition of the biosynthesis of photosynthetic pigments in *A. arguta* leaves compared to monoplanting and, conversely, an increase in their content in apple leaves (Table 1). Photosynthetic characteristics can reflect a plant‘s photosynthetic capacity, physiological and ecological patterns of adaptation to different environments, and light utilization strategies Throughout the growth process, plants optimize light energy utilization by adjusting chlorophyll content and ratio (*a*/*b*), which allows plants to continuously adapt to the environment (Li et al., 2010).

In particular, the content of chlorophyll *a* in *A. arguta* leaves in the variants of the experiment in the ratio of 50 : 50%, 67 : 33%, and 33 : 67% decreased by 1.5 times, respectively, compared to monoplanting, and the content of chlorophyll *b* and carotenoids, respectively, by 1.1–1.4 and 1.1–1.2 times. For *M. domestica* plants, the concentration of chlorophyll *a* increased by 1.2–2.1 times compared to monoplanting, and the content of chlorophyll *b* increased by 1.1–1.8 times. In the distribution of carotenoids, the opposite dependence can be traced, with the exception of the variant in which the ratio between *M. domestica* and actinidia plants was 50 : 50%. The increase in the content of carotenoids in *M. domestica* leaves indicates the activation of the adaptive potential of plants (Table 1). Photosynthetic characteristics can reflect a plant‘s

Table 1 Content of photosynthetic pigments in the leaves of *Actinidia arguta* under conditions of joint planting with *Malus domestica* (2020–2021), ($mg \cdot kg^{-1}$ FW)

Variants	*a*	*b*	k	*a* + *b*	*a*/*b*	(*a* + *b*)/k
A. arguta	0.106	23	0.040	0.130	4.55	3.26
***A. arguta* 50% – *M. domestica* 50%**	0.070	19	0.039	0.090	3.68	2.31
***A. arguta* 67% – *M. domestica* 33%**	0.073	21	0.032	0.094	3.46	2.92
***A. arguta* 33% – *M. domestica* 67%**	0.070	16	0.035	0.087	4.28	2.49
LSD 0.05	0.00014	0.00013	0.00013	0.0001	1.15	0.51
M. domestica	0.081	0.049	0.082	0.130	1.67	1.59
***M. domestica* 50% – *A. arguta* 50%**	0.097	0.073	0.085	0.170	1.32	1.99
***M. domestica* 67% – *A. arguta* 33%**	0.169	0.053	0.057	0.223	3.17	3.90
***M. domestica* 33% – *A. arguta* 67%**	0.168	0.087	0.053	0.255	1.92	4.82
LSD 0.05	0.00020	0.00021	0.0003	0.00020	1.54	1.54

Notes: a – chlorophyll *a*; b – chlorophyll *b*; k – carotenoids; LSD – Least Significant Difference

Table 2 Content of photosynthetic pigments in the leaves of *Actinidia arguta* under conditions of joint planting with *Schisandra chinensis* (2020–2021), (mg·kg^{-1} FW)

Variants	***a***	***b***	**k**	***a + b***	***a/b***	***(a + b)/*k**
A. arguta	0.106	0.023	0.040	0.130	4.55	3.26
***A. arguta* 50% – S.chinensis 50%**	0.090	0.023	0.015	0.113	3.88	7.42
***A. arguta* 67% – S.chinensis 33%**	0.100	0.022	0.039	0.122	4.49	3.12
***A. arguta* 33% – S.chinensis 67%**	0.087	0.021	0.036	0.108	4.05	3.00
LSD 0.05	0.0001	0.00013	0.0013	1.37	1.15	0.51
S. chinensis	0.069	0.022	0.032	0.092	3.12	2.88
S.chinensis 50% – *A. arguta* 50%	0.048	0.016	0.018	0.065	2.84	3.45
S.chinensis 67% – *A. arguta* 33%	0.056	0.020	0.028	0.076	2.78	2.68
S.chinensis 33% – *A. arguta* 67%	0.039	0.023	0.023	0.062	1.68	2.62
LSD 0.05	0.00018	0.0017	0.00019	0.00027	0.202	0.32

Notes: a – chlorophyll *a*; b – chlorophyll *b*; k – carotenoids; LSD – Least Significant Difference

photosynthetic capacity, physiological and ecological patterns of adaptation to different conditions (Li et al., 2010).

A similar pattern can be observed in an experiment of growing *A. arguta* with *Sch. chinensis* (Table 2). In all variants of the experiment, the suppression of the biosynthesis of photosynthetic pigments in the leaves of plants under the conditions of joint plantings was revealed. In particular, the concentration of chlorophyll *a* in the leaves decreased by 7–19% according to the experimental variants compared to the monoplanting of actinidia. The decrease in the content of chlorophyll *b* and carotenoids was in the range of 4–8% and 2–64%, respectively. For magnolia vine plant tissues these values were 19–28% for chlorophyll *a*, 11–24% for chlorophyll *b*, and 10–41% for carotenoids, respectively. This makes the application of nondestructive reflectance spectroscopy to assess the physiological state of plants very promising. In fact, knowledge of the ratio between carotenoid and chlorophyll content in leaves could provide more useful information for determining the physiological state of plants than knowledge of the absolute amounts of individual pigments, as it may ignore differences between plant species and cultivars and provide more accurate indications of plant senescence (Demmig-Adams et al., 1996).

When *A. arguta* was grown together with dogwood, the opposite dependence was revealed, namely: the content of photosynthetic pigments in both *A. arguta* leaves and dogwood leaves increased, especially in the variant with a ratio of 33 : 67% (Table 3). The concentration of chlorophyll *a* in *A. arguta* and *C. mas* leaves increased 1.1–1.4 times, chlorophyll *b* 1.6–1.9 times, and carotenoids 1.3 times.

According to the results obtained in the variants with *A. arguta* and *C. japonica*, significant fluctuations in the content of photosynthetic pigments in *A. arguta* leaves

Table 3 Content of photosynthetic pigments in the leaves of *Actinidia arguta* under conditions of joint planting with *Cornus mas* (2020–2021), (mg·kg^{-1} FW)

Variants	***a***	***b***	**k**	***a + b***	***a/b***	***(a + b)/*k**
A. arguta	0.106	0.023	0.040	0.130	4.55	3.26
***A. arguta* 50% – *C. mas* 50%**	0.067	0.024	0.023	0.092	2.78	3.92
***A. arguta* 67% – *C. mas* 33%**	0.063	0.023	0.035	0.086	2.68	2.46
***A. arguta* 33% – *C. mas* 67%**	0.117	0.038	0.051	0.156	3.04	3.02
LSD 0.05	0.00018	0.00020	0.00019	0.00072	0.33	0.28
C. mas	0.104	0.023	0.036	0.127	4.37	3.55
***C. mas* 67% – *A. arguta* 33%**	0.105	0.035	0.035	0.140	2.98	4.00
***C. mas* 50% – *A. arguta* 50%**	0.103	0.040	0.035	0.144	2.52	4.08
***C. mas* 33% – *A. arguta* 67%**	0.142	0.048	0.045	0.191	2.93	4.20
LSD 0.05	0.00017	0.0020	0.00020	3.71	1.74	2.15

Notes: a – chlorophyll *a*; b – chlorophyll *b*; k – carotenoids; LSD – Least Significant Difference were found, in particular, the level of chlorophyll *a* in mixed planting ranged from 0.019 mg/to 0.100 mg·kg^{-1} FW and chlorophyll *b* from 0.018 to 0.050 mg·kg^{-1} FW (Table 4). For this reason, the ratio of carotenoids to chlorophylls is commonly used to analyze the physiological status of plants (Demmig-Adams et al., 1996). Thus, accurate and reliable estimation of the carotenoid to chlorophyll ratio is in itself an important goal for understanding plant physiological processes.

Certain differences can be traced concerning the ratio of the amount of chlorophylls to the content of carotenoids, which indicate the physiological state of plants and serve as indicators of stress. During the joint cultivation of *A. arguta* plants and *M. domestica*, this indicator is significantly lower for *A. arguta* leaves compared to monoculture, which proves the fact that the development of *A. arguta* is inhibited. At the same time, for *M. domestica* plants, on the contrary, this ratio is higher, especially in variants with *M. domestica* 67%, *A. arguta* 33% and *M. domestica* 33%, *A. arguta* 67%. This is confirmed by the high content of chlorophyll *b* in *M. domestica* leaves the increase in which concentration is associated with the stimulation of growth processes in plants. Thus, it can be noted that under the joint cultivation of *A. arguta* and *M. domestica* trees, there is inhibition of the biosynthesis of photosynthetic pigments in *A. arguta* plants and stimulation in *M. domestica* trees. The analysis of the data obtained proves the importance of assessing the content of photosynthetic pigments and analyzing changes in their activity to obtain real information about the physiological state of plants, as noted in some works (Sims and Gamon, 2002).

Regarding the results obtained under the conditions of the simultaneous cultivation of *A. arguta* and *Sch. chinensis* plants, it was found that there are no significant differences in the ratio of the sum of chlorophylls and carotenoids, except for the option *A. arguta* 50% – *Sch. chinensis* 50%. The resulting dependence indicates a sufficiently low compatibility of these fruit cultures. The ratio of the sum of chlorophylls to carotenoid content has proven to be the most indicative indicator trait under conditions of joint cultivation of plants, which is used to determine the impact of a stress factor (Li et al., 2010).

In the joint plantings of *A. arguta* and *C. mas*, the following dependence is observed: the maximum indicators are characteristic of actinidia plants at the ratio of *A. arguta* 50% – *C. mas* 50%, and for *C. mas* plants at the ratio of *C. mas* 33% – *A. arguta* 67%. At the same time, insignificant suppression of the photosynthetic activity of *A. arguta* plants was observed in the presence of *C. mas*.

In variants with *C. japonica*, the highest value of the ratio between the sum of chlorophylls and carotenoids was recorded for planting *A. arguta* 33% – *C. japonica* 67% and for monoplanting *C. japonica*. In general, actinidia plants had a positive effect on the development of *C. japonica* plants, which is manifested in the induction of the biosynthesis of photosynthetic pigments.

Usually, when plants are under stress, the content of individual photosynthetic pigments (chlorophylls and carotenoids) and the ratio between them changes accordingly, with chlorophylls being characterized by a tendency to a faster decrease in activity compared to carotenoids (Gitelson et al., 1994). Experimentally confirmed the negative relationship between the content of photosynthetic pigments in the leaves of the experimental plant species and the concentration of nitrate nitrogen. At the same time, the ratio of

Table 4 Content of photosynthetic pigments in the leaves of *Actinidia arguta* under the conditions of joint planting with *Chaenomeles japonica* (2020–2021), mg·kg^{-1} FW

Variants	*a*	*b*	k	*a + b*	*a/b*	*(a + b)/*k
A.arguta	0.106	0.023	0.040	0.130	4.55	3.26
***A. arguta* 50% – *C. japonica* 50%**	0.019	0.050	0.035	0.070	0.39	1.99
***A. arguta* 67% – *C. japonica* 33%**	0.071	0.018	0.026	0.089	3.82	3.45
***A. arguta* 33% – *C. japonica* 67%**	0.100	0.019	0.029	0.119	5.09	4.13
LSD 0.05	0.00011	0.00017	0.00019	0.00015	0.23	1.93
C. japonica	0.159	0.076	0,039	0.235	2.09	5.94
***C. japonica* 50% – *A. arguta* 50%**	0.187	0.044	0,051	0.231	4.20	4.54
***C. japonica* 67% – *A. arguta* 33%**	0.162	0.081	0,046	0.244	2.00	5.27
***C. japonica* 33% – *A. arguta* 67%**	0.202	0.082	0.069	0.284	2.47	4.09
LSD 0.05	0.00085	0.0002	0.00017	0.0003	0.08	0.12

Notes: a – chlorophyll *a*; b – chlorophyll *b*; k – carotenoids; LSD – Least Significant Difference.

Table 5 Nitrate nitrogen content in the leaves of experimental plant species under the conditions of joint cultivation (mg·kg^{-1} FW)

NO$_3$ content	**Variants**							
	A. arguta	***A. arguta* 50% – *M. domestica* 50%**	***A. arguta* 67% – *M. domestica* 33%**	***A. arguta* 33% – *M. domestica* 67%**	***M. domestica***	***M. domestica* 50% – *A. arguta* 50%**	***M. domestica* 67% – *A. arguta* 33%**	***M. domestica* 33% – *A. arguta* 67%**
	68.33±3.42	96.87 ±4.81	94.21 ±4.56	97.15 ±4.79	95.24 ±4.32	75.09 ±3.56	55.72 ±2.63	57.58 ±2.68
	A. arguta	***A. arguta* 50% –** *S. chinensis* **50%**	***A. arguta* 67% –** *S. chinensis* **33%**	***A. arguta* 33% –** *S. chinensis* **67%**	*S. chinensis*	*S. chinensis* **50% – *A. arguta* 50%**	*S. chinensis* **67% – *A. arguta* 33%**	*S. chinensis* **33% – *A. arguta* 67%**
	68.33 ±3.42	87.53 ±4.21	72.18 ±3.55	91.24 ±4.43	117.21 ±5.36	136.58 ±6.11	125.06 ±5.83	169.72 ±7.95
	A. arguta	***A. arguta* 50% – *C. mas* 50%**	***A. arguta* 67% – *C. mas* 33%**	***A. arguta* 33% – *C. mas* 67%**	***C. mas***	***C. mas* 50% – *A. arguta* 50%**	***C. mas* 67% – *A. arguta* 33%**	***C. mas* 33% – *A. arguta* 67%**
	68.33 ±3.42	146.51 ±6.91	149.14 ±7.09	65.81 ±3.08	69.11 ±3.18	68.69 ±3.28	68.91 ±3.18	61.39 ±2.94
	A. arguta	***A. arguta* 50% – *C. japonica* 50%**	***A. arguta* 67% – *C. japonica* 33%**	***A. arguta* 33% – *C. japonica* 67%**	***C. japonica***	***C. japonica* 50% – *A. arguta*** 50%	***C. japonica* 67% – *A. arguta* 33%**	***C. japonica* 33% – *A. arguta* 67%**
	68.32 ±3.42	211.18 ±9.94	140.19 ±6.83	69.07 ±3.31	59.25 ±2.84	48.29 ±2.33	55.93 ±2.61	45.13 ±2.11

chlorophylls increased with a decrease in leaf nitrogen content, so this trait can serve as an indicator of N distribution (Terashima and Hikosaka 1995; Sims and Gamon, 2002). Different forms of nitrogen affect the overall balance of nitrogen distribution of this element in the leaves, ensuring high efficiency of its use (Wei et al., 2022).

In the second stage of research, the peculiarities of the distribution of nitrate nitrogen in the leaves of fruit crops were analyzed under the conditions of simultaneous cultivation. Among different nitrogen sources, the nitrate form is mostly taken up by root systems and transported to pagonia (Crawford, 1995; Lam et al., 1996).

A negative relationship between the content of photosynthetic pigments, especially chlorophyll *a*, in the leaves of the experimental plant species and the content of nitrate nitrogen in the tissues was revealed (Table 5, Figure 1–4). According to the theory of optimal nitrogen distribution, the ratio of chlorophylls increases with decreasing nitrogen content in the leaves (Evans, 1989).

The availability of nitrogen in plants increased in mixed plantings, while the greatest value was observed in the variant of the experiment *A. arguta* 33% – *M. domestica* 67% (97.15 mg·kg^{-1} FW) (increase compared to control by 42%). Environmental stresses that occur under the influence of various factors lead to a decrease in photosynthetic activity (Demming et al., 1996; Li et al., 2010). Regardless of the cause of changes in leaf nitrogen concentration, there is always a positive correlation between nitrogen concentration and the photosynthetic capacity of plants (Field and Mooney, 1986).

In monoculture *M. domestica* plants, the nitrogen content in the leaves was lower compared to the conditions of joint cultivation, in particular, in the variant *M. domestica* 67% – *A. arguta* 33%, a decrease in its concentration by 70% compared to the control. In the version of *A. arguta* and *S. chinensis*, the highest level of nitrate nitrogen in leaves was recorded compared to the monoculture. In addition to more effective management strategies through fertilization, the concentration of nitrate in plants can be changed by adjusting growth conditions, thereby promoting the conversion of nitrate to organic compounds (Bian et al., 2015; Hickman, 2016; Rogers et al., 2020).

During the joint cultivation of *A. arguta* – *C. mas*, a similar pattern was revealed, in particular, the accumulation of nitrogen under these conditions, compared to monoplanting.

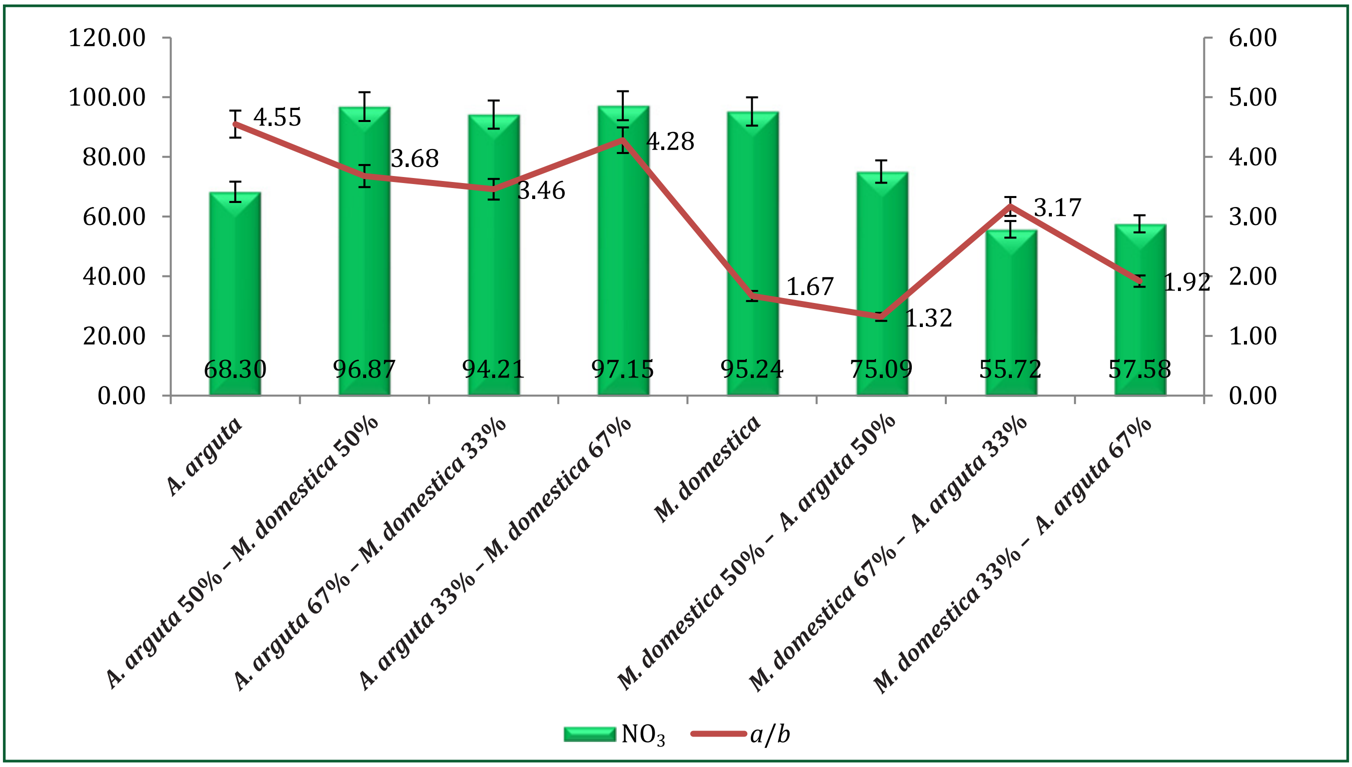

Figure 1 Distribution of nitrogen in the leaf and the ratio of chlorophyll (Chl) *a*/*b* in the leaves of experimental plant species *Actinidia arguta*, *Malus domestica* under the conditions of joint cultivation (mg·kg^{-1})

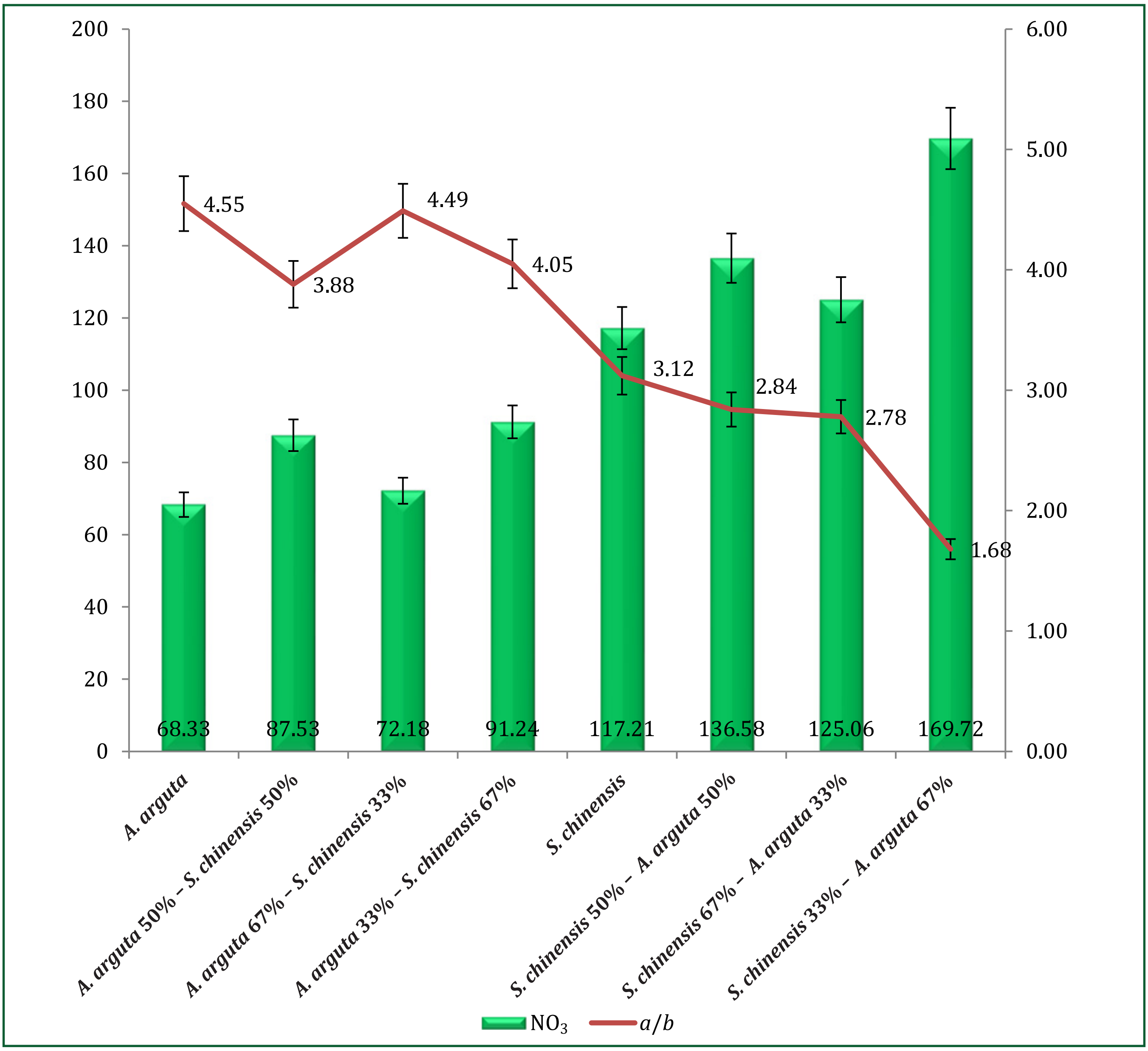

Figure 2 Distribution of nitrogen and the ratio of chlorophylls *a*/*b* in the leaves of the plant species *Actinidia arguta*, *Schisandra chinensis* under the conditions of joint cultivation (mg·kg^{-1})

At the same time, the highest value of nitrate nitrogen was recorded in the variant *A. arguta* 67% – *C. mas* 33% – 149.14 mg·kg^{-1} FW (118% increase). *C. mas* plants in mixed plantings did not significantly decrease the amount of nitrogen compared to monoculture. In the variant *A. arguta* – *C. japonica*, the accumulation of nitrogen in *A. arguta* leaves was also detected under joint cultivation compared to monoculture, the highest value was observed in *A. arguta* 50% – *C. japonica* 50% – 211.18 mg·kg^{-1} FW (by 209%). Among the various sources of nitrogen, the nitrate form is mostly taken up by root systems and transported to shoots (Crawford, 1995; Lam et al., 1996). The uptake, assimilation and translocation of nitrate in plants are regulated by many internal signals (expression of related genes and enzyme activity), as well as environmental factors (Xu et al., 2012).

C. japonica plants in mixed stands showed a slight decrease in the amount of nitrogen compared to the control. The data presented in the figures are a visual confirmation of the dependence we discovered.

At the same time, the highest value of nitrate nitrogen was recorded in the variant *A. arguta* 67% – *C. mas* 33% – 149.14 mg·kg^{-1} FW (118% increase). Intensive nitrogen uptake by plants has a positive effect on the photosynthetic activity of plants (Vries et al., 2016; Prinsi and Espen, 2018). In addition to more effective

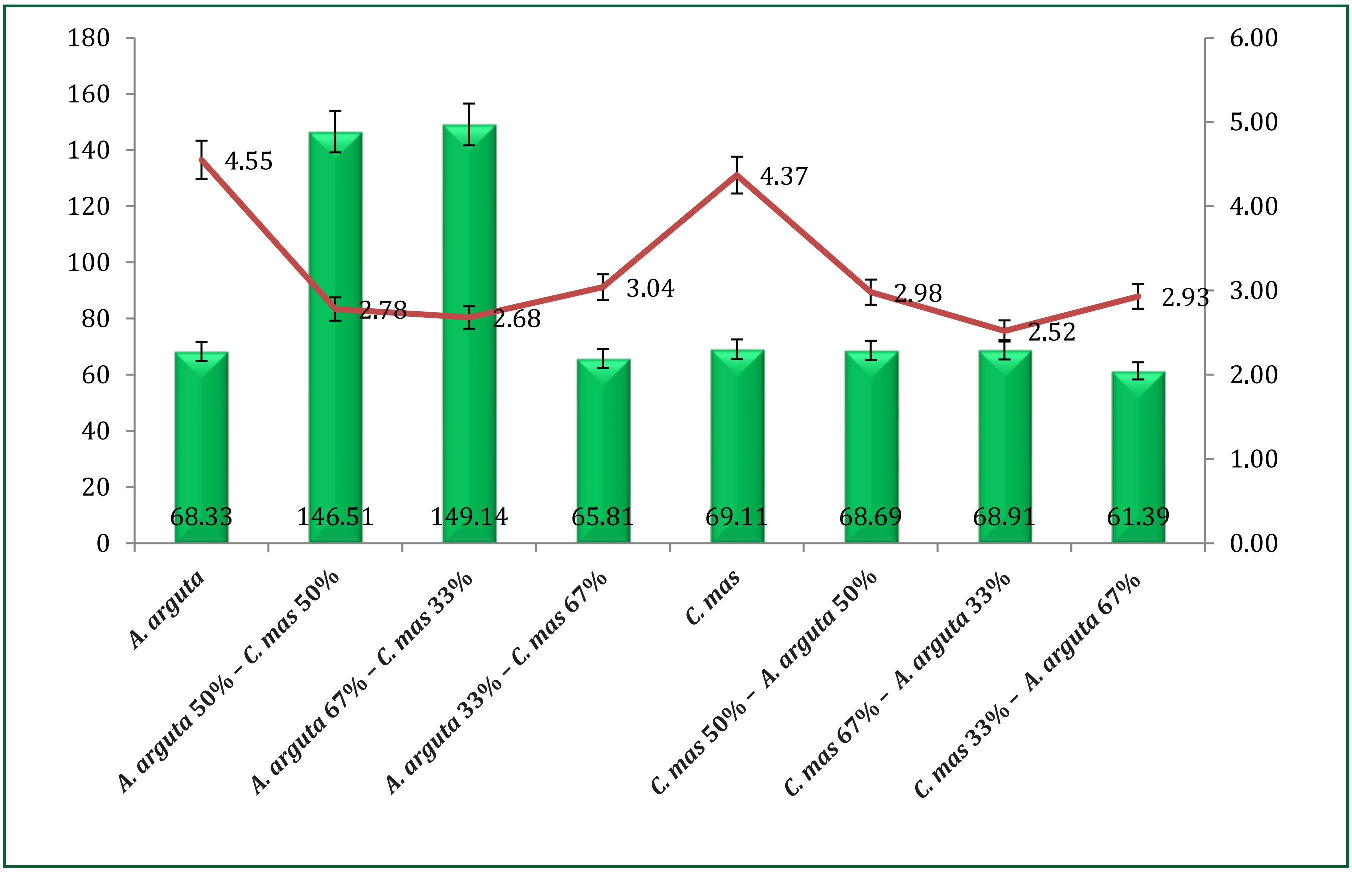

Figure 3 Distribution of nitrogen and the ratio of chlorophylls *a*/*b* in the leaves of experimental plant species *A. arguta* and *C. mas* under the conditions of joint cultivation (mg·kg^{-1})

management strategies through fertilization, the concentration of nitrate in plants can be changed by adjusting growth conditions, thereby promoting the conversion of nitrate to organic compounds (Bian et al., 2015, Hickman, 2016; Rogers et al., 2020). *C. mas* plants in mixed plantings did not significantly decrease the amount of nitrogen compared to monoculture. In the variant *A. arguta* – *C. japonica*, the accumulation of nitrogen in *A. arguta* leaves was also detected under joint cultivation compared to monoculture, the highest value was observed in *A. arguta* 50% – *C. japonica* 50% – 211.18 mg·kg^{-1} FW (by 209%).

Efficient N uptake and utilization can significantly affect the intensity of plant N consumption, thereby affecting overall photosynthetic efficiency and productivity (Evans, 1989; Arvin, 2019; Evans and Clarke, 2019). *C. japonica* plants in mixed stands showed a slight decrease in the amount of nitrogen compared to the control. The data presented in the figures are a visual confirmation of the dependence we discovered. One of the explanations for this dependence may be the allelopathic activity of plants, which manifests itself in blocking membrane permeability, water and nutrient absorption, respiration, photosynthesis, protein, and nucleic acid synthesis, and plant growth regulation (Latif et al., 2017). It can be assumed that it is plant allelochemicals that cause a negative impact on the growth and development of other crops in compatible plantings (since the effect of allelochemicals on plant photosynthesis mainly includes inhibition or damage to the mechanisms of synthesis and acceleration of the decomposition of photosynthetic pigments (Mbagwu, 2006; Yu et al., 2006; Sahoo et al., 2010).

Conclusions

It was established that the photosynthetic activity of plants and the content of nitrate nitrogen are important indicators that illustrate the physiological state of plants under different growing conditions. The indicator of the ratio of the sum of chlorophylls to carotenoids is the most indicative for use in assessing the compatibility of species in mixed plantings. According to these indicators, *C. mas* plants proved to be the most suitable for growing together with *A. arguta*, while *S. chinensis* cannot grow together with this culture in mixed plantings. The obtained data can be used in further physiological, ecological, and allelopathic investigations of different fruit plants.

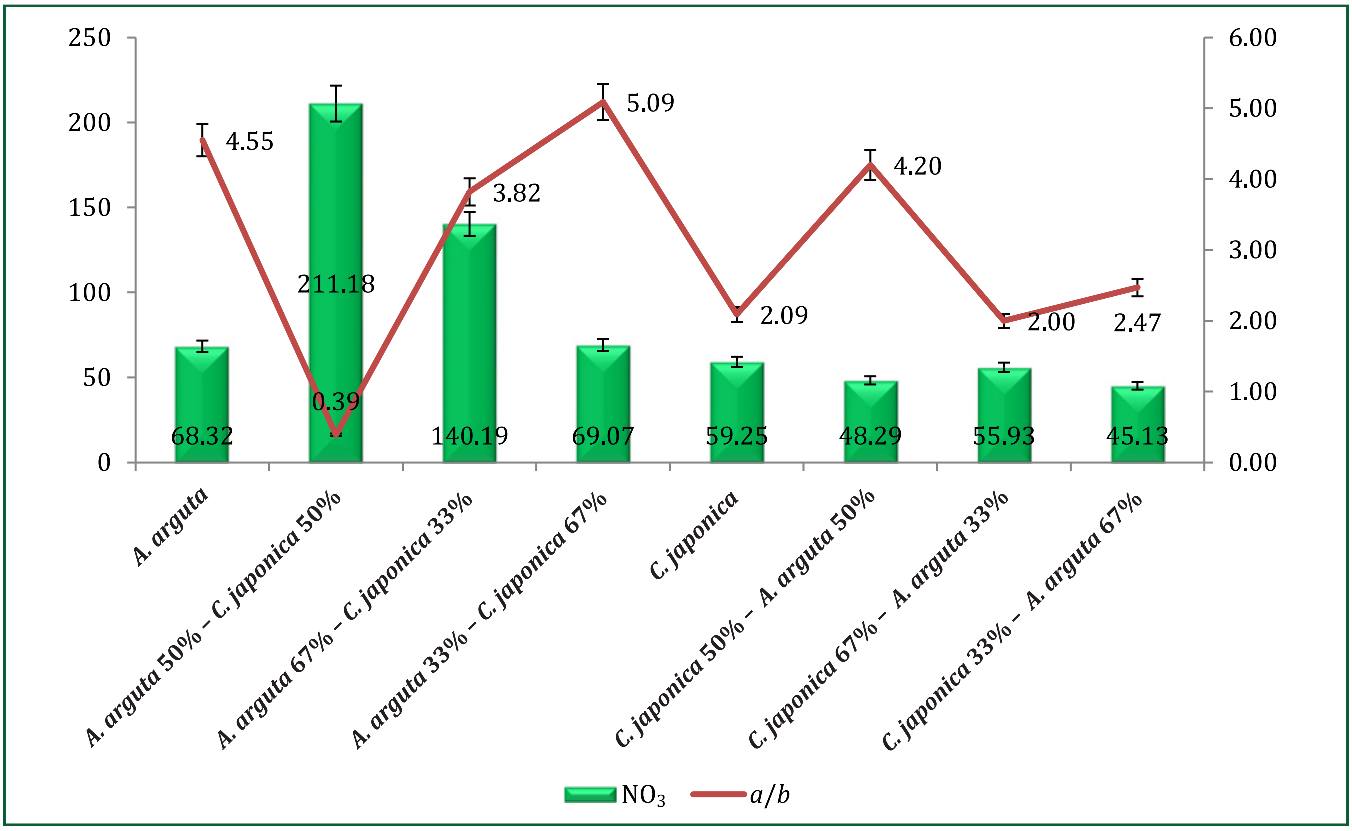

Figure 4 Distribution of nitrogen and the ratio of chlorophylls *a/b* in the leaves of experimental plant species *Actinidia arguta*, *Chaenomeles japonica* under the conditions of joint cultivation (mg·kg^{-1})

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article does not contain any studies that would require an ethical statement.

References

Arvin, P. 2019. Study of Different levels of nitrogen, phosphorus and potassium on physiological and morphological parameters and essential oils in savory plant (*Satureja hortensis* L.). In *Journal of Plant Research (Iranian Journal of Biology)*, 32(2), 260–279.

Bian, Z.H., Yang, Q.C., & Liu, W.K. 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: A review. In *Journal of the Science of Food and Agriculture*, 95, 869–877. https://doi.org/10.1002/jsfa.6789

Calzadilla, P.I., Carvalho, F.L., Gomez, R., Neto, L., & Signorelli, M.C. 2022. Assessing photosynthesis in plant systems: A cornerstone to aid in the selection of resistant and productive crops. In *Environmental and Experimental Botany*, 201(9). https://doi.org/10.1016/j.envexpbot.2022.104950

Crawford, N.M. 1995. Nitrate: Nutrient and signal for plant growth. In *Plant Cell*, 7, 859.

Demmig-Adams, B., & William, W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. In *Trends in Plant Science*, 1, 21–26.

Driever, S., Simkin, A., Alotaibi, S., Fisk, S., Madgwick, P., Sparks, C., Jones, H., Lawson, T., Parry M., & Raines, C. 2017. Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. In *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372. https://doi.org/10.1098/rstb.2016.0384

Evans, J.R. 1989. Partitioning of nitrogen between and within leaves grown under different irradiances. In *Journal of Plant Physiology*, 16, 533–548.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. In *Oecologia*, 78, 9–19. https://doi.org/10.1007/BF00377192

Evans, J.R., & Clarke, V.C. 2019. The nitrogen cost of photosynthesis. In *Journal of Experimental Botany*, 70(1), 7–15. https://doi.org/10.1093/jxb/ery366

Fahad, S., Bajwa, A., Nazir, U., Shakeel, A., Anjum, S., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M., Alharby, H., Wu, C., Wang W., & Huang, J. 2017. Crop production under drought and heat stress. In *Plant Responses and Management Optionse*, 8, 1147. https://doi.org/10.3389/fpls.2017.01147

Fathi, A., & Zeidali, E. 2021. Conservation tillage and nitrogen fertilizer: a review of corn growth, yield andweed management. In *Central Asian Journal of Plant Science Innovation*, 1(3), 121–142.

Field, C., & Mooney, H.A. 1986. The photosynthesis-nitrogen relationship in wild plants. In T. Givnish (ed). In *On the Economy of Plant Form and Function*. Cambridge University Press, 25–55.

Gitelson, A.A., & Merzlyak, M. 1994. Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. Leaves. Spectral features and relation to chlorophyll estimation. In *Journal of Plant Physiology,* 143(3), 286–292. https://doi.org/10.1016/S0176-1617(11)81633-0

Hickman, G.A. 2016. *Review of Currently Available Data on the International Production of Vegetables in Greenhouses.* Cuesta roble greenhouse consultants; Mariposa, CA, USA: 2016. International greenhouse vegetable production-statistics.

Hikosaka, K. 2004. Interspecific difference in the photosynthesis-nitrogen relationship: patterns, physiological causes, and ecological importance. In *Journal of Plant Research*, 117, 481– 494. https://doi.org/10.1007/s10265-004-0174-2

Hikosaka, K., & Terashima, I. 1995. A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. In *Plant, Cell and Environment,* 18, 605– 618. https://doi.org/10.1111/j.1365-3040.1995.tb00562.x

Hitchcock, A., Hunter, C., Sobotka, R., Komenda, J., Dann, M., & Leister, D. 2022. Redesigning the photosynthetic light reactions to enhance photosynthesis – the PhotoRedesign consortium In *Plant J*, 109(1) 23–34. https://DOI: 10.1111/tpj.15552

Huang, J., Hadimani, S.B, & Rupon, J.W. 2002. Iron nitrosyl hemoglobin formation from the reactions of hemoglobin and hydroxyurea.In *Biochemistry*, 41, 2466–2474.

Hubbart, S., Smillie, I., Heatley, M., Swarup, R., Chuan Ching Foo, Liang Zhao, & Murchie, E. 2018. Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. In *Communications Biology*, 1. https://DOI: 10.1038/s42003-018-0026-6

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S., Iwai, M. Niyogi, K., & Long S. 2016. Improving photosynthesis and crop productivity by accelerating recovery from. In *Science*, 18, 354(6314), 857–861. https://DOI: 10.1126/science.aai8878

Lam, M., Coschigano, K.T., Oliveira, I.C., Melo-Oliveira, R., & Coruzzi, G.M. 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. In *Annual Review of Plant Biology*, 47, 569–593.

https://doi: 10.1146/annurev.arplant.47.1.569

Latif, S., Chiapusio, G., Latif, S., Chiapusio, G., & Weston, L.A. 2017. Allelopathy and the role of allelochemicals in plant defence. In *Advances in Botanical Research*, 82, 1–54. https://doi.org/10.1016/bs.abr.2016.12.001

Li, X., Zheng, J.C., Yan, S.H., Ren, C.G., Wang, M., & Cong W. 2010. Dynamics of photosynthesis in eichhornia crassipes solms of jiangsu of China and their influencing factors. In *African Journal of Biotechnology,* 9, 7302–7311. https://doi:10.5897/AJB10.1087

Li, Y., He, N.P., Hou, J.H., Xu, L., Liu, C., & Zhang J.H. 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. In *Frontiers in Ecology and Evolution,* 6. https://doi:10.3389/fevo.2018.00064

Lichtenthaler, H.K., & Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. In *Biochemical Society Transaction*, 11(5), 591–592. https://doi.org/10.1042/bst0110591

Mbagwu, F., 2006. The allelopathic effects of crude water extracts of *Annona muricata* on common weed. In *International Journal of Natural and Applied Sciences,* 2(4), 341–344.

Mitchell, M., Pritchard, J., Okada, S., Zhang, J., Venables, I., Vanhercke, T., & Jean-Philippe Ral. 2020. Increasing growth and yield by altering carbon metabolism in a transgenic leaf oil crop. In *Plant Biotechnology Journal,* 18, 2042. https://doi.org/10.1111/pbi.13363

Moeinirad, A., Zeinali, A., Galeshi, S., Afshin, S., & Eganepour, F. 2021. Investigation of fluorescence chlorophyll sensitivity, chlorophyll index, rate of Chlorophyll (*a*, *b*), nitrogen concentration and nitrogen nutrition index under under nitrogen and phosphorus nutrition in wheat. In *Journal of Crop Production,* 14(1), 1–18. https://doi.org/10.22069/ejcp.2021.12259.1947

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., & Villar, R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta analysis. In *New Phytologist*, 182(3), 565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Prinsi, B., & Espen, L. 2018. Time-course of metabolic and proteomic responses to different nitrate/ammonium availabilities in roots and leaves of maize. In *International Journal of Molecular Sciences*, 19, 2202. https://doi: 10.3390/ijms19082202

Rinkis, G. Y., & Nolendorf, V. F. 1982. *Balanced plant nutrition micro and microelements*. Riga. «Zinatna».

Rogers, A., Kumarathunge, D. P., Lombardozzi, D. L., Medlyn, B. E., Serbin, S.P. & Walker, A.P. 2020. Triose phosphate utilization limitation: an unnecessary complexity in terrestrial biosphere model representation of photosynthesis. In *New Phytologist*, 230. https://doi: 10.1111/nph.17092

Sahoo, U.K, Jeeceelee, L., Vanlalhriatpuia, K., Upadhyaya, K., & Lalremruati, J.H. 2010. Allellopathic effects of leaf leachate of *Mangifera indica* L. on initial growth parameters of few home garden food crops. In *World Applied Sciences Journal,* 10(12), 1438–1447.

Scavo, A., Restuccia, A., & Mauromicale, G. 2018. Allelopathy: principles and basic aspects for agroecosystem control. In Gaba, S., Smith, B., Lichtfouse, E. (eds) Sustainable Agriculture Reviews. In *Sustainable Agriculture Reviews*, 28. Springer, Cham. https://doi.org/10.1007/978-3-319-90309-5_2

Simkin, A., Patricia, E., Calcagno, L., Davey, P., Headland, L., Lawson T., Timm, S., Bauwe, H., & Raines, C.A. 2017. Simultaneous stimulation of sedoheptulose 1, 7-bisphosphatase, fructose 1, 6-bisphophate aldolase and the photorespiratory glycine decarboxylase H protein increases CO_2 assimilation, vegetative biomass and seed yield in *Arabidopsis*. In *Plant Biotechnology Journal*, 15. https://doi.org/10.1111/pbi.12676

Sims, D.A., & Gamon, J. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. In *Remote Sens of Environment*, 81 (2–3), 337–354. https://doi.org/10.1016/S0034-4257(02)00010-X

Vries, F.T., Brown, C., & Stevens, C.J. 2016. Grassland species root response to drought:consequences for soil carbon and nitrogen availability. In *Plant Soil*, 409, 297–312. https://doi: 10.1007/s11104-016-2964-4

Wang, C.M., Chen, H.T., Li, T.C., Weng, J.H., Jhan, Y.L., & Lin, S.X. 2014. The role of pentacyclic triterpenoids in the allelopathic effects of Alstonia scholaris. In *Journal of Chemical Ecology*, 40, 90–98. https://doi: 10.1007/s10886-013-0376-y

Wei, X., Yang, Y., Yao, J., Han, J., Yan, M., & Zhang J. 2022. Improved utilization of nitrate nitrogen through within-leaf nitrogen allocation trade-offs in *Leymus chinensis*. In *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.870681

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Byron, B., Lamont, B.B., Lee, T., Lee, W., & Villar, R. 2004. The worldwide leaf economics spectrum. In *Nature*, 428(6985), 821–827. https://doi.org/10.1038/nature02403

Xu, G., Fan, X., & Miller A.J. 2012. Plant nitrogen assimilation and use efficiency. In *Annual Review of Plant Biology,* 63, 153–182. https://doi: 10.1146/annurev-arplant-042811-105532

Yu, J.H., Zhang, Y., Niu, C.X., & Li, J.J. 2006. Effects of two kinds of allelochemicals on photosynthesis and chlorophyll fluorescence parameters of *Solanum melongena* L. seedlings. In *Chinese Journal of Applied Ecology*, 17, 1629–1632.

Zar, J.H. 1999. *Biostatistical Analysis*. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.