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POLLEN GRAIN EXPRESSION OF INTRINSIC AND OSMOLYTE INDUCED OSMOTIC ADJUSTMENT IN A SET OF WHEAT CULTIVARS

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ABSTRACT

Osmotic adjustment is one of the important traits, potentially useful for improving plant performance under drought. We tested pollen response to osmotic stress, produced by immersion in 55% or 65% PEG 6000 solutions, without or with addition of KCl, to estimate osmotic adjustment (OA), by using quantitative measurements of projected pollen cytoplasm area, in 43 wheat cultivars. We found large differences between cultivars, both for the effect of PEG solutions without KCl addition ("intrinsic OA") and for the effect of adding KCl to the PEG solutions ("osmolyte induced OA"). The total (overall) OA depended almost equally on the two OA components. The intrinsic and the osmolyte induced OA were not correlated, and this suggests that combining high levels of both mechanisms by breeding might allow genetic progress in drought resistance.

Key words: osmotic adjustment (OA), intrinsic OA, osmolyte induced OA, wheat.

INTRODUCTION

Among the many mechanisms and traits, potentially useful for improving plant performance under drought (Blum, 1996, 1998; Ginkel et al., 1998), osmotic adjustment (OA) has received wide recognition as a major mechanism of drought resistance in crop plants (Zhang et al., 1999). Osmotic adjustment capacity, which allows maintaining the cell turgour by accumulation of organic or anorganic osmolytes as result of increasing water stress, is one of the important characteristics to adapt to water deficit, and is the only one to be activated only under stress, and therefore less likely to affect yield potential in non-stress conditions (Morgan, 1983, 1999). As osmotic adjustment is a cellular mechanism, it is expressed in all plants cell, including pollen grain and this offers a convenient way to characterize germplasm for this trait (Morgan, 1999; Moud and Yamagishi, 2005).

Patil and Ravikumar (2011) in their work on osmotic adjustment in sorghum differentiated two types of osmotic adjustment:

- the intrinsic OA, estimated by the ratio between the pollen area under osmotic stress and the initial area;
- the OA induced by accumulation of anorganic cations, estimated by the change in pollen area following addition of an anorganic osmolyte to the stressing solution.

Previous research at NARDI Fundulea identified large differences among some wheat cultivars in pollen response to osmotic stress (Bănică et al., 2008; David, 2009). This paper presents results on estimation of intrinsic and induced OA, using quantitative measurements of pollen grain response, in a larger and more diverse wheat collection.

MATERIAL AND METHODS

A very diverse collection of forty three winter and spring *Triticum aestivum* and *Triticum durum* cultivars was studied for pollen expressed osmotic adjustment, using the test of pollen developed by Morgan (1999).

This collection included:

– 16 Romanian cultivars with various performance under drought, from Izvor described as having best drought resistance among Romanian wheat cultivars to Fundulea 4, adapted to a more humid climate (Mustăţea et al., 2009; Săulescu et al., 2006);

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– 3 near-isogenic lines developed at Texas A&M University, characterized as different in their performance under drought (Balotă et al., 2008);

– 2 *Triticum aestivum* and 3 *Triticum durum* spring cultivars grown on significant acreage in various regions of Algeria, under diverse conditions of water stress (Boufenar and Zaghouan, 2006; Younes, 2009);

– Plainsman V., an old US cultivar, and 00X0090-54, a Kansas breeding line, described as drought resistant (Farshadfar et al., 2001; Sears, R.G., *personal communication*);

– several cultivars from Hungary, Serbia and Moldova, assembled in a collection for studying stress resistance, in the frame of SEE-ERA.NET project.

Wheat plants were grown in the field at the National Agricultural Research and Development Institute Fundulea, Romania, in 2011.

Moderate water stress was present at the time when spikes at anthesis were collected. Pollen grains of matured anthers, collected from these spikes, were soaked in polyethylene glycol (PEG 6000) solutions of 55% or 65% concentration, over microscope slides, with or without 10 mM KCl added to solutions. After a little agitation to release the pollen grains, the anther sections were removed and the solution covered with a cover slip. Slides were incubated at 20°C for 2 days.

Microscopic observations were made using a magnification of 100X and 200X. Pollens grains are usually spherical to ellipsoidal in shape. A stressing concentration of PEG induced shrinkage of pollen grains, which assumed a more conical shape, often with concavities. Modification of pollen grains shape (shrinking) was estimated by measuring the projected area of pollen cytoplasm.

The following parameters were recorded (Patil and Ravikumar, 2011):

A – Initial size. Because when pollen grains are immersed in water they absorb it and burst, the initial size was considered the size measured after 48 hours of immersion in a non-stressing solution of 30% PEG.

B – The projected size of the cytoplasm after 48 hours of osmotic stress induced by 55% or 65% PEG solutions.

C – The projected size of the cytoplasm after 48 hours of incubation in 55% or 65% PEG solutions with 10 mM KCl added to solutions.

Measurements were made using Quickphoto2.3 (program Olympus).

RESULTS AND DISCUSSION

There were large differences in the projected area of cytoplasm in pollen grains subjected to various levels of osmotic stress in PEG solutions, among the studied wheat cultivars (Table 1). Projected cytoplasm area of pollen grain cytoplasm soaked in 55% and 65% PEG solutions, averaged over all cultivars, was smaller than the initial average area (measured in the non-stressing 30% PEG solution), by 18.1% and 21.5% respectively. Adding KCl to the PEG solutions, increased the area of projected pollen cytoplasm on average by 5.1% for the 55% PEG solution and by 4.9% for the 65% PEG solution.

Despite the relatively large standard deviations calculated for some cultivars, which express the inherent high variability of pollen grains, it is obvious that cultivars reacted differently to the applied osmotic stress treatments.

As seen in Figure 1, in some cultivars projected area of cytoplasm did not change (in line TX86A8072), or even increased (in cultivar Izvor) after immersion in PEG solutions, while in others the area decreased by more than 30% (in cultivar Apache). Addition of KCl in the PEG solutions greatly increased (in line TX86A8072 or Miranda), or barely changed the cytoplasm area (in cultivars Izvor and Apache). This suggests that different cultivars might use different types of osmotic adjustment, with different levels of involvement of anorganic cations.

According to Patil and Ravikumar (2011), the response of pollen projected area to osmotic stress induced by PEG can be considered as „intrinsic” osmotic adjustment, while the response to external supply of osmolyte is described as „induced” OA.

Table 1. Cytoplasm projected area of pollen subjected to osmotic stress, with or without addition of KCl

Cultivar	Projected area of pollen cytoplasm (µm²) after exposure to solutions of:				
	PEG 30% (A)	PEG 55% (B_1)	PEG 65% (B_2)	PEG 55%+KCl (C_1)	PEG 65%+KCl (C_2)
00X0090-54	2713.2±202.6	2072.2±339.3	2319.6±264.5	2250.3±316.9	1887.0±164.5
Ain Abid	2633.0±289.3	2081.2±199.9	1859.5±249.5	1874.5±114.3	2205.5±316.2
Alex	2450.7±152.9	2215.7±291.4	2215.7±221.7	1618.0±185.9	1808.2±378.8
Apache	3009.2±207.5	2457±117.9	1515.7±103.5	2648.0±408.6	1557.3±119.0
Apullicum	2221.0±288.4	1530.5±108.2	1950.8±137.4	1506.5±76.8	1477.2±245.5
Arieşan	2541.6±296.5	2047.5±213.3	2447.2±305.0	2818.6±175.0	2542.3±431.1
Balada	3412.5±261.8	2431.5±152.6	2379.8±347.1	3396.0±329.6	2415.7±228.5
Bankuti 1201	3301±335.3	1865.2±161.8	1481.2±92.8	3072.5±316.8	1811.5±222.4
Bidi17	2619.3±302.2	1475.7±314.6	2055.0±263.1	1889.7±206.1	2687.5±145.4
Ceres	2330.8±318.4	1857.3±87.1	1374.8±259.3	2121.8±348.3	1844.3±198.5
CMSS99Y03439	2180.0±173.0	2026.8±128.1	2161.7±125.4	2032.0±181.0	2211.3±96.7
Dacia	2534.0±149.3	1758.2±215.0	1710.0±243.6	2062.2±152.5	1855.3±258.7
Dropia	2569.5±251	1805.2±157.3	1759.3±488.6	1736.5±149.4	1927.3±376.2
Elida	2824.8±184.6	2080.8±182.4	2517.0±112.0	2458.2±174.8	1965.8±256.7
Evropa 90	2260.3±254.1	1897.3±122.7	1765.2±136.8	2091.5±147.3	2932.3±258.0
F00030 G	1988.7±91.2	2737.8±143.5	1810.5±118.0	1977.0±173.3	2085.2±184.9
F05503 G	2910.7±287.9	2131.8±225.5	2073.5±433.3	2261.5±218.1	2111.8±409.9
Flamura 85	2866.6±199.4	2198.5±313.5	2968.8±134.6	2210.7±289.6	3939.2±394.1
Fundulea 4	3275.2±221.1	2369.2±358.3	2066.3±167.4	2262.3±181.8	2331.3±175.5
Gergana	2236.8±108.1	1802.0±294.4	1631.8±207.2	1510.5±156.6	1539.8±207.9
Giura 31-4	3203.7±267.4	1820.5±281.8	1660.7±222.5	1667.8±149.3	1572.5±229.8
Hiddab	2863.5±202.3	2881.3±260.4	1892.5±317.3	2366.7±150.0	2396.5±72.8
Izvor	2505.8±290.7	2699.2±215.0	3838.0±327.4	3096.5±208.6	3153.2±427.6
Jiana	3085.2±168.7	2337.3±220.6	1742.0±173.5	2414.5±128.7	1668.0±267.3
Litera	3685.8±348.3	2356.7±216.1	2999.0±449.9	2741.7±347.6	2285.0±187.3
Lovrin 34	2353.7±205.6	3247.2±289.5	2177.7±169.8	2047.5±200.9	2146.7±245.0
M. B. Bachir	2458.2±332.7	1953.3±126.4	1930.8±245.3	2966.3±208.6	3028.3±319.8
Milenka	2707.0±319.7	2584.7±350.6	2037.3±265.2	3186.3±281.1	3408.7±304.9
Miranda	3075.5±195.6	2271.5±173.1	2809.0±345.5	1880.0±110.0	1813.5±62.4
Monada	2874.0±92.7	2075.7±98.2	1890.2±246.4	2165.3±216.3	1658.5±176.9
Murga	2708.7±275.8	2696.8±360.6	2317.5±168.2	2788.5±237.6	2505.3±206.1
MV 16	2719.7±117.5	2485.2±121.1	2425.3±198.1	2753.8±108.5	2172.0±184.7
MV.Magdalena	2547.0±126.9	2690.5±153.7	1823.3±239.7	2504.0±95.6	1993.0±208.1
MV. Mazurka	2646.3±192.7	2752.7±139.2	1906.2±226.0	2860.7±191.3	2038.0±384.0
MV. Taborzo	3030.2±299.4	2265.5±246.1	3045.3±883.4	2024.0±83.9	2271.2±126.2
Plainsman V.	3431.2±216.8	2329.8±198.0	2302.7±300.4	3024.0±296.8	2995.7±210.1
Pobeda	3038.4±63.1	2723.2±213.0	2341.5±236.9	2754.2±142.9	2257.3±112.9
Radika	3113.0±216.2	2347.2±161.0	1946.0±234.7	1522.3±334.9	2022.5±226.5
Skopjanka	2845.2±176.2	2117.0±132.3	2489.2±329.4	2105.0±85.2	2018.0±161.8
TX86A5606	3040.8±306.7	2148.0±247.9	2259.8±401.2	2940.7±253.2	1948.8±317.5
TX86A8072	2158.3±194.5	2294.7±208.5	2063.0±149.3	2586.5±213.6	4142.8±319.6
TX88A6880	1840±112.9	2200.7±163.6	1963.7±148.0	2719.7±241.5	2032.5±147.3
Vitron	2612.7±227.0	2014.0±109.3	2254±269.8	2145.3±217.5	2010.0±94.5
Average	2730.76	2235.67	2143.67	2350.22	2248.22

Based on this concept, we analysed cultivar response to osmotic stress, using:

- the ratios of projected pollen area under stress (average of both 55% and 65% PEG without KCl) on non-stressed initial area (B/A), as a measure of intrinsic OA;
- the ratios of pollen area under stress with external osmolyte supply (average of 55% and 65% PEG with KCl addition) on the average of the 55% and 65% PEG without KCl (C/B), as a measure of induced OA;
- the ratios of pollen area under stress with external osmolyte supply (average of 55% and 65% PEG with KCl addition) on non-stressed initial area (C/A), as a measure of total (overall) OA.

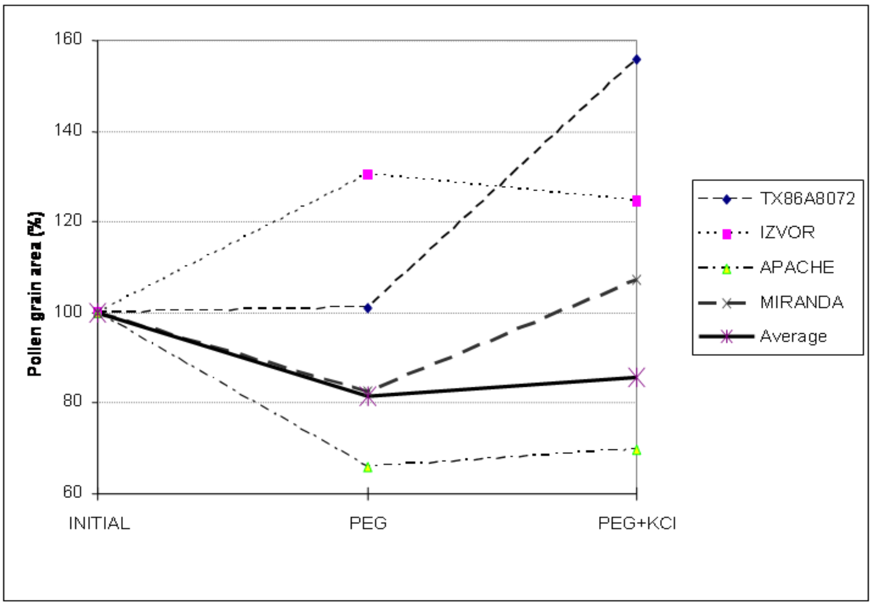

Figure 1. Cultivar response of pollen cytoplasm projected area to osmotic stress (induced on average by 55% and 65% PEG solutions) with or without osmolyte addition

Large differences were found among tested cultivars for all types of osmotic adjustment (Table 2). For intrinsic OA, the limits of variation were from 0.507 in Bankuti 1201 to 1.304 in Izvor.

Although performance under water stress depends on many plant characters and physiological traits, it should be noticed that the cultivars with highest intrinsic osmotic adjustment have been previously described as drought resistant. This suggests that this trait plays an important role in the adaptation to water stress.

Adding KCl to PEG solutions to which pollen grains were exposed produced a change in the shape and size, either by restoring the initial size, or even increasing it, or reducing at some extent. The ratio of projected pollen area under stress with external osmolyte supply (average of 55% and 65% PEG with KCl addition) on the average of the same PEG solutions without KCl (C/B) varied among cultivars from 0.773 in Alex to 1.568 in TX86A8072.

The cultivar response to adding osmolytes to stress PEG solutions was not correlated with their intrinsic osmotic adjustment (Figure 2). Only a few cultivars (cultivar Izvor and lines TX86A8072 and TX88A6880) expressed both types of OA, while other cultivars only had one type, or had low values for both types.

This suggests that the intrinsic and osmolyte induced OA might be independent mechanisms that might be combined by breeding, for obtaining improved behaviour under water stress.

The total osmotic adjustment capacity, estimated by the ratio of pollen area under stress with external osmolyte supply and the non-stressed initial area (ratio C/A), integrates both the intrinsic and the osmolyte induced OA. It varied from 0.506 in Giura 31-4 to 1.559 in TX86A8072.

Table 2. Differences between cultivars in intrinsic, osmolyte induced and total osmotic adjustment, as expressed in pollen grains

Classif.	Cultivar	Intrinsic osmotic adjustment B/A	Induced osmotic adjustment C/B	Total osmotic adjustment C/A
1.	TX86A8072	1.010±0.534	1.568±0.537	1.559±0.353
2.	TX88A6880	1.132±0.160	1.135±0.125	1.291±0.385
3.	Izvor	1.304±0.035	0.984±0.003	1.247±0.445
4.	Evropa 90	0.810±0.260	1.382±0.318	1.111±0.035
5.	Milenka	0.854±0.218	1.317±0.260	1.107±0.075
6.	Flamura 85	0.901±0.142	1.166±0.117	1.073±0.145
7.	Miranda	0.826±0.207	1.308±0.247	1.072±0.046
8.	Arieşan	0.884±0.139	1.208±0.156	1.055±0.098
9.	F00030 G	1.144±0.120	0.937±0.071	1.021±0.253
10.	Murga	0.926±0.025	1.058±0.013	0.977±0.125
11.	CMSS99Y03439	0.961±0.009	1.013±0.026	0.973±0.152
12.	MV. Mazurka	0.880±0.013	1.054±0002	0.926±0.076
13.	MV 16	0.903±0.026	1.002±0.047	0.906±0.092
14.	Lovrin 34	1.152±0.259	0.808±0.199	0.891±0.211
15.	MV. Magdalena	0.886±0.035	1.012±0.040	0.883±0.062
16.	Plainsman V.	0.675±0.144	1.299±0.213	0.877±0.143
17.	Bidi 17	0.674±0.141	1.294±0.207	0.874±0.142
18.	Balada	0.705±0.092	1.206±0.124	0.852±0.103
19.	Ceres	0.693±0.101	1.242±0.158	0.851±0.129
20.	Hiddab	0.834±0.040	1.044±0.016	0.832±0.011
21.	Pobeda	0.833±0.047	0.988±0.073	0.825±0.021
22.	TX86A5606	0.725±0.027	1.116±0.037	0.804±0.089
23.	Vitron	0.817±0.062	0.978±0.085	0.795±0.002
24.	Elida	0.814±0.071	0.981±0.082	0.783±0.016
25.	Ain Abid	0.748±0.023	1.043±0.031	0.775±0.068
26.	Dacia	0.684±0.031	1.129±0.044	0.773±0.129
27.	00X0090-54	0.809±0.088	0.950±0.115	0.762±0.015
28.	F05503 G	0.722±0.023	1.040±0.039	0.751±0.089
29.	Mohamed B. Bachir	0.790±0.082	0.951±0.117	0.751±0.027
30.	Bankuti 1201	0.507±0.153	1.435±0.320	0.740±0.374
31.	Skopjanka	0.809±0.126	0.903±0.162	0.725±0.020
32.	Dropia	0.694±0.037	1.029±0.055	0.713±0.119
33.	MV. Taborzo	0.876±0.200	0.820±0.234	0.709±0.021
34.	Fundulea 4	0.677±0.034	1.042±0.045	0.701±0.140
35.	Apache	0.660±0.022	1.053±0.037	0.699±0.150
36.	Alex	0.904±0.234	0.773±0.275	0.699±0.044
37.	Litera	0.727±0.096	0.963±0.116	0.682±0.105
38.	Gergana	0.768±0.132	0.891±0.180	0.682±0.055
39.	Apullicum	0.784±0.156	0.871±0.198	0.672±0.051
40.	Monada	0.690±0.081	0.960±0.124	0.665±0.120
41.	Jiana	0.661±0.060	0.995±0.094	0.662±0.148
42.	Radika	0.690±0.177	0.844±0.241	0.569±0.135
43.	Giura 31-4	0.543±0.113	0.932±0.177	0.506±0.259

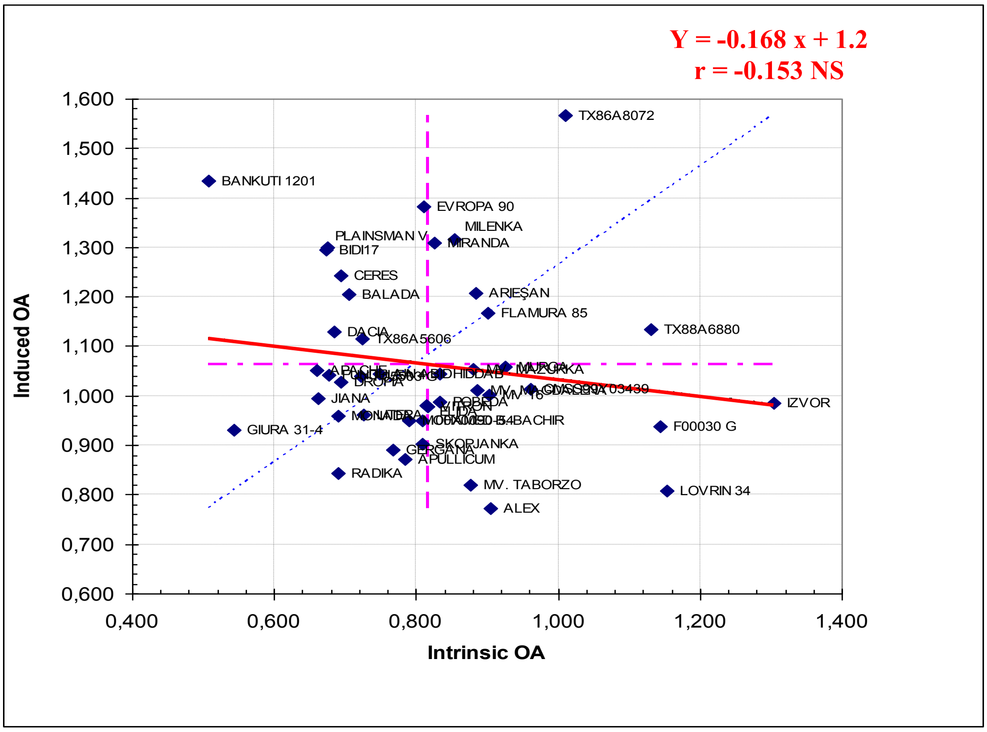

Figure 2. Relationship between intrinsic and osmolyte induced osmotic adjustment

The total OA was closely corelated with the intrinsic OA, which explained about 43% of its variation (Figure 3), but also with the osmolyte induced OA, which explained about 36% of total OA variation (Figure 4).

Morgan (1999) stated that the pollen expression of osmotic adjustment only occurred after addition of potassium chloride to the stressing PEG solution, as a result of the effect of the osmoregulation gene on potassium transport. Our data suggest that besides this mechanism, the intrinsic OA, which does not require the presence of an external supply of osmolyte, can greatly differentiate cultivars, having an important contribution to the total OA. According to Patil and Ravikumar (2011), the intrinsic OA might be explained by biochemical changes and/or pre-synthesized biochemicals.

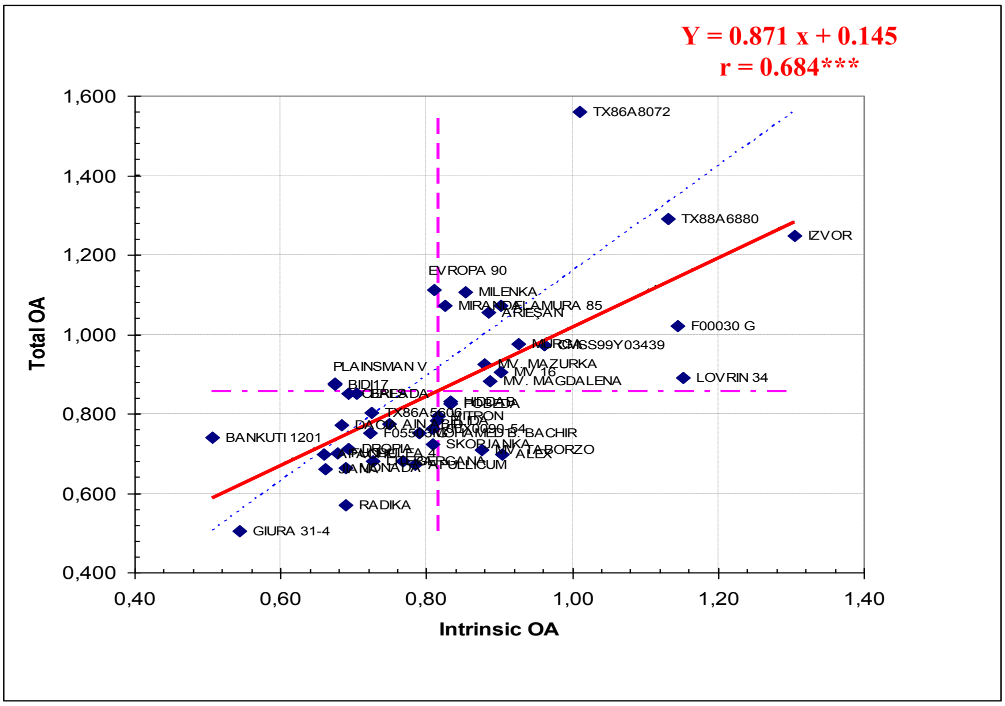

Figure 3. Relationship between intrinsic OA and total OA

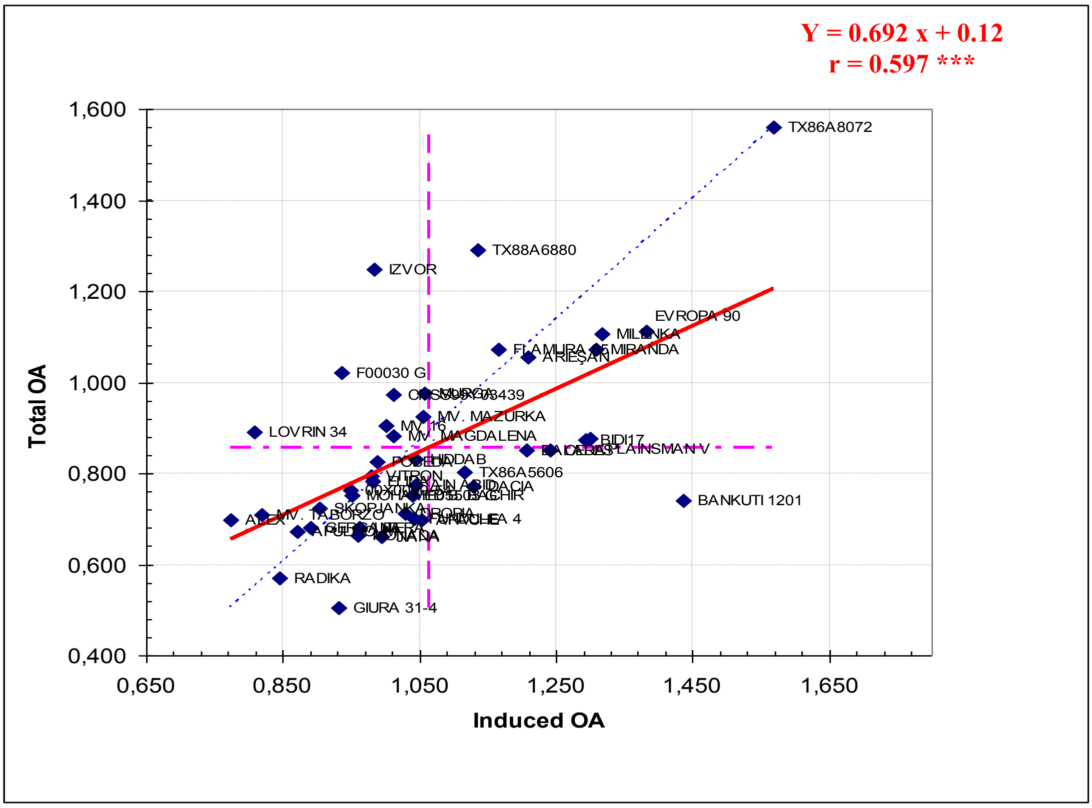

Figure 4. Relationship between osmolyte induced OA and total OA

CONCLUSIONS

We found large differences between cultivars in two types of osmotic adjustment, which involve or not the presence of an external supply of osmolytes. The intrinsic and the induced OA contributed almost equally to the variation of overall OA. As the intrinsic and the osmolyte induced OA were not significantly correlated, combining high levels of both mechanisms by breeding might allow genetic progress in drought resistance.

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