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**NOTICE OF RELEASE OF LETTUCE BREEDING
LINES RH08-0472 AND RH-08-0475**

Iceberg lettuce breeding lines with resistance to *Verticillium* wilt caused by race 1 isolates of *Verticillium dahliae*.

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Executive Summary

The Agricultural Research Service, United States Department of Agriculture and the University of California, Davis, announce the release of two breeding lines of lettuce (*Lactuca sativa* L.). Lines RH08-0472 and RH08-0475 are F₉ iceberg type lettuce breeding lines with resistance to *Verticillium* wilt caused by *V. dahliae*. They were selected from the cross ‘Tiber’ x (‘La Brillante’ x ‘Pacific’). Resistance is derived from ‘La Brillante’, a Batavia type lettuce cultivar with resistance to race 1 isolates of *V. dahliae*. These breeding lines are suitable for commercial production in *V. dahliae*-infested and non-infested fields. They are being released for commercialization of *Verticillium* wilt iceberg cultivars and for use as parents in further breeding of resistant cultivars. They have been deposited in the National Plant Germplasm System as W6 39944 (RH08-0472) and W6 39945 (RH08-0475).

Introduction

Verticillium wilt, caused by the soil borne fungus *Verticillium dahliae* Kleb., is a serious soil borne disease affecting a broad range of economically important crops (Pegg and Brady, 2002). Lettuce (*Lactuca sativa* L) was not considered a host for *V. dahliae* until the mid 1990s, when the disease was discovered in coastal production districts of California (Subbarao et al, 1997). The disease has subsequently spread within coastal California (Atallah et al. 2010), and has also been detected on lettuce in the Mediterranean basin (Garibaldi et al. 2007; Ligoxigakis et al. 2002). *Verticillium dahliae* is seed transmitted in lettuce and other vegetable crops grown in rotation with lettuce, raising concerns regarding its spread to other lettuce production areas (Atallah et al. 2010; Vallad et al. 2005). While all types of lettuce are susceptible, *Verticillium* wilt is most damaging to iceberg type cultivars. Plants often remain symptomless until they near harvest maturity, at which time the symptoms develop quickly. Basal or “wrapper leaves” that completely cover the outer part of the head wilt, and then collapse as the disease progresses, leading to premature plant death and an unharvestable head. Other key foliar symptoms include stunting, defoliation and other developmental abnormalities. Prior to the onset of foliar symptoms, the vascular discoloration of root and stem tissues, revealed by vertical sectioning of the plant, are the only other diagnostic features.

Host resistance is the best long term control method in lettuce, since current cultural control methods are cost prohibitive, potentially damaging to the environment, or of limited feasibility (Subbarao et al. 1997). Two pathogenic races (race 1 and race 2) of *Verticillium dahliae* on lettuce have been described. The Batavian cultivar La Brillante and several other heirloom cultivars are resistant to race 1 isolates, while no known source of resistance to race 2 isolates has been reported (Hayes et al. 2007; Vallad et al. 2006). Race 1 resistance in 'La Brillante' is complete (no symptom development) (Vallad and Subbarao 2008), and continues to be effective in grower fields (Hayes et al. 2007). We are reporting on the development of iceberg breeding lines RH08-0472 and RH08-0475, which have resistance to race 1 isolates of *V. dahliae* inherited from 'La Brillante'.

Origin and Development

RH08-0472 and RH08-0475 were selected from the cross 'Tiber' x 01-1071; 01-1071 was an F₅ line derived from 'Pacific' x 'La Brillante'. Breeding line 01-1071 is notable because it is the source line from which the *Verticillium* wilt resistant breeding lines RH05-0336, RH05-0339, and RH05-0340 were selected and released in 2006 (Hayes et al. 2006). RH05-0336, RH05-0339, and RH05-0340 were the first iceberg lines bred for *Verticillium* wilt resistance, but were not suitable for commercial production. 'La Brillante' is a yellow-green Batavia type cultivar that is not used for commercial production in California. The origin of 'La Brillante' is unknown, but has been characterized as highly susceptible to the calcium deficiency related disorder tipburn (data not shown, see Ryder, 1999 for a review of tipburn in lettuce), partially resistant to the disease big-vein caused by the soil borne virus *Mirafiori lettuce big-vein virus* (Ryder and Robinson, 1995), resistant to the disease dieback caused by the soil borne viruses in the *Tombusviridae* family (Simko et al. 2009), and has a short shelf-life after processing into salad (Hayes and Liu, 2008). 'Tiber' and 'Pacific' are modern iceberg type cultivars developed by the USDA and are broadly adapted for coastal California production conditions. 'Pacific' was specifically selected for resistance to big-vein disease (Ryder and Robinson, 1991), while 'Tiber' was developed with improved resistance to tipburn (Ryder and Waycott, 1998). 'Pacific', and 'Tiber' are susceptible to *Verticillium* wilt (Hayes et al. 2007).

Lettuce ($2n=2x=18$) is an autogamous species and all seed was produced by allowing greenhouse grown plants to self-pollinate; seed harvested from individual plants was kept separate unless otherwise noted. The general breeding strategy for developing *Verticillium* wilt resistant icebergs was to select between families for resistance to *Verticillium* wilt, and then between and within resistant families for superior horticultural characteristics. All evaluations and selections were conducted in infested grower field experiments located in Watsonville, CA. The race structure of *V. dahliae* is not precisely known in these sites, but the sites are believed to be predominantly infested with race 1 strains (Hayes et al. 2007). However, the existence of some race 2 isolates has been established at these sites (Maruthachalam et al. 2010). Resistance evaluations were conducted by uprooting ten randomly selected plants from test plots of 40 to 100 plants, vertically sectioning the root and crown and assessing the plant for root discoloration and foliar symptoms typical of *Verticillium* wilt. Single plant selections with industry acceptable iceberg type head characteristics were subsequently taken from only the families deemed resistant (typically < 10% disease incidence), and repotted in an isolated greenhouse for seed production. Selection within families for resistance was not technically possible, since the assay is destructive. However, wilting plants with *Verticillium* like foliar symptoms were avoided.

RH08-0472 and RH08-0475 are derived from a single F₅ plant developed using the breeding scheme described above. This plant belonged to a line that appeared to be fixed for resistance beginning in the F₄ generation. In 2007, a F_{5:7} seed lot was developed by massing several F₆ plants grown under non-*V. dahliae*-infested conditions, and had limited testing for adaptation in 2008 as breeding line RH07-0341. No disease testing was conducted in 2007 and 2008, as all *V. dahliae* infested field sites were either fumigated or unavailable. Regardless, slight variations in maturity were noticed among F₇ plants in a Salinas, CA non-infested field experiment, and single plant selections were made to obtain the F_{7:8} lines RH08-0472 and RH08-0475. Adaptation and disease resistance characterization were subsequently conducted in 2009 and 2010 using F_{7:8} and F_{7:9} seed respectively that was produced in non-*V. dahliae* infested field sites.

Description

Characterization of RH08-0472 and RH08-0475 in field experiments was largely done in comparison to ‘Salinas 88’, a cultivar developed from backcrossing the *mol*² allele for *Lettuce mosaic virus* resistance into the cultivar Salinas (Ryder, 1991). RH08-0472 and RH08-0475 closely resemble ‘Salinas 88’, as well as ‘Tiber’ and ‘Pacific’ (Pictures of RH08-0472: <http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=W6+39944>; Pictures of RH08-0475: <http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=W6+39945>). The outer leaves are dull green while the internal leaves are light yellow. Leaves are wider than they are long with wavy margins, moderate savoy and minimal undulation. The apex is truncate to emarginate or notched. Heads are firm at maturity, slightly wider than tall, and occasionally oblong when viewed from the top. Ribs are flat to occasionally protruding. Days to market maturity, initiation of bolting, anthesis, and seed set are equivalent to ‘Salinas 88’. Seeds are black.

Disease resistance, Horticultural characteristics, Yield, and Post harvest quality

Field experiments, data collection, and methods. Adaptation, yield, horticultural characters, post-harvest quality and resistance to physiological defects were assessed in field experiments having diverse locations and planting dates. Resistance to the diseases Verticillium wilt, big-vein, and downy mildew (caused by the Oomycete *Bremia lactucae*) was assessed in multiple replicated field experiments or greenhouse experiments. ‘Salinas 88’, the breeding lines parent’s ‘Pacific’ and ‘Tiber’, as well as known check cultivars were included in experiments as needed. Production conditions for field experiments were standard for coastal California (Ryder, 1999), except where specified.

The Verticillium wilt resistance of RH08-0472 and RH08-0475 was assessed in four replicated experiments at a field site infested solely with a race 1 isolate (VdLs16) of *V. dahliae* located in Salinas, CA. Resistance to race 2 isolate VdLs17 was also assessed in a single replicated greenhouse experiment using the methods of Hayes et al. (2007). In all experiments, Verticillium wilt disease incidence (DI) was assessed using the methods of Hayes et al. (2007).

Verticillium wilt DI, horticultural characteristics, and yield was also assessed in two unreplicated infested experiments conducted in grower field sites in Salinas, CA (Trials 1 and 2 in Tables 1 and 2). The exact *V. dahliae* race structure is unknown at these locations, but is believed to be predominated by race 1 strains based on previous field experiments (Hayes and Subbarao, unpublished results). Yield was assessed as percent harvestable heads and subsequently transformed to cartons per acre. The horticultural characters assessed include head weight (g), head height (cm), head diameter (cm), core height (cm), and maturity (1 = immature

through 5 = over mature), and percent tipburn. Data were collected on two evaluation dates separated by one week.

Using field sites not infested with *V. dahliae*, two replicated experiments in Soledad, CA (trials 3 and 4 on table 2) and two replicated experiments located at the USDA research farm in Salinas, CA (trials 5 and 6 on table 2) were conducted to assess yield, horticultural characteristics, and tipburn resistance using the method and variables previously discussed. In addition, post-harvest quality of whole heads and minimally processed salad was assessed using lettuce harvested from the USDA research farm. Shelf-life of minimally processed salad was evaluated using the methods of Hayes and Liu (2008) and rated on a scale of 0% decay to – 100% decay. The quality of whole heads after shipment to markets (arrival quality) was assessed for Salinas 88 and RH09-0472. RH09-0475 was not included due to an insufficient number of heads. Twenty-four heads (pooled from all plots) per breeding line or cultivar were harvested on August 10, 2010, packed in cartons, vacuum cooled, and transported in a refrigerated truck to Beltsville, MD. Upon arrival the heads were stored at 5 °C, and each head was rated by three trained evaluators on August 24, 2010 using a five point scale with zero corresponding to no apparent decay and four corresponding to greater than 75% decay. Each head was treated as a replicate for data analysis.

The incidence of big vein symptomatic plants was recorded in the Soledad, CA field experiments, and resistance to downy mildew in two replicated field experiments in Salinas, CA. Big-vein resistance was assessed as the percentage of big-vein symptomatic plants, while downy mildew severity was assessed on a scale of 0 = no disease through 5 = severe disease.

Statistical analysis of data from replicated experiments was conducted using PROC Mixed of SAS (Version 9.2; SAS Institute, Inc., Cary, NC) following the recommendations of Littell et al. (2006). Mean comparisons were adjusted using the Tukey option. Percent big-vein data was analyzed on the arcsine scale, and backtransformed and reported on tables as disease rating (DR). Verticillium DI and DS data was analyzed using ranked data in the PROC Mixed procedure in SAS (2004, version 9.2) (Brunner et al. 2002; Shah and Madden 2004); relative effects (RE) and 95% confidence intervals for comparison of cultivars and breeding lines were calculated using the LD_CI macro as done with other Verticillium wilt resistance experiments (Hayes et al. 2007). The line x experiment interaction was not significant for replicated Verticillium wilt field experiments, so the data were pooled into a single analysis. No statistical comparisons were conducted with unreplicated data.

Verticillium wilt resistance. RH08-0472 and RH08-0475 have levels of resistance to Verticillium wilt comparable to ‘La Brillante’, while ‘Salinas 88’ and ‘Tiber’ are highly susceptible. In four replicated field experiments infested with race 1 *V. dahliae* isolate VdLs16, the disease incidence of ‘La Brillante’, RH08-0472 and RH08-0475 was significantly lower compared to ‘Salinas 88’ and ‘Tiber’ (Table 1). In two unreplicated infested grower field experiments, no root or foliar symptoms of Verticillium wilt were observed in ‘La Brillante’, RH08-0472 or RH08-0475 (Table 1, trials 1 and 2). In the susceptible cultivar ‘Salinas 88’, disease incidence was 80% and 100% in trials 1 and 2 respectively (Table 1).

Yield and horticultural performance in infested and non-infested field experiments. RH08-0472 or RH08-0475 produce commercially acceptable yields with good horticultural characteristics when grown in infested or non-infested fields, and have a range of adaptation to production methods and harvest dates equivalent to ‘Salinas 88’. In two unreplicated field experiments conducted in *V. dahliae* infested grower fields in Salinas, CA (Table 2, trials 1 and 2), yield, head weight, head height, and head width were generally unchanged or increased for

RH08-0472 and RH08-0475 between the first and second harvest dates (Table 2, trials 1 and 2). In contrast, these same variables were generally unchanged or decreased for ‘Salinas 88’, as a result from the onset of *Verticillium* wilt symptoms. For all lines, core height and maturity generally increased from first to second harvest date.

In four replicated field experiments conducted in non-infested fields located in Soledad, CA and Salinas, CA and with harvest dates ranging from late April to early August, yields (% harvestable and cartons per acre) were generally similar to ‘Salinas 88’ and the other commercially acceptable cultivars that were tested (Table 2, trials 3 – 6). In a single experiment in Salinas, CA, RH08-0475 had yields significantly lower than ‘Salinas 88’. The experiment was highly unusual due to the relatively advanced maturity of ‘Salinas 88’ and late maturity of RH08-0475 combined with an undetermined bottom rot that prevented the option of a later harvest date. Regardless, this experiment demonstrates that environments may exist that can result in reduced yields for at least RH08-0475. RH08-0472 or RH08-0475 may have the potential for slightly larger and heavier heads compared to ‘Pacific’ and ‘Salinas 88’. In two experiments harvested in April, head weights were significantly greater than ‘Pacific’ and ‘Salinas 88’. Head height, head width, core height, and maturity were not significantly different from the commercial cultivars tested.

Tipburn resistance and post-harvest quality. Tipburn incidence was recorded in two infested and four non-infested field experiments. On the 9.16.2010 harvest date of unreplicated trial 1, the tipburn incidence was 100%, 10%, 20%, and 30% for ‘La Brillante’, RH08-0472, RH08-0475, and ‘Salinas 88’ respectively (Table 2). In trials 5 and 6, the tipburn incidence was not significantly different between the breeding lines and the check cultivars Tiber, Pacific, and Salinas 88 (Table 2). No tipburn was observed in trials 2, 3 and 4 (Table 2). The shelf-life of minimally processed salad of RH08-0472, RH08-0475, and ‘Salinas 88’ were not significantly different (Table 2). However, ‘Salinas 88’, RH08-0472, and RH08-0475 were significantly better than the susceptible controls ‘Triple Threat’ (romaine lettuce) and ‘La Brillante’ (Table 2). Considering the tipburn resistance and shelf-life of RH08-0472 and RH08-0475, these lines appear to be suitable for use in the minimally processed salad market. In a single field experiment, the whole head arrival quality of RH08-0472 was significantly lower compared to Salinas 88, a cultivar with exceptional arrival quality (E.J. Ryder, personal communication) (Table 2). These results indicate that commercial production of RH08-0472 and RH08-0475 should use methods that will ensure the highest possible arrival quality of whole heads.

Resistance to big-vein disease and downy mildew. The resistance to big-vein in RH08-0472, and RH08-0475 is similar to ‘Salinas 88’ and ‘Tiber’, which in these field experiments was not significantly different than ‘Pacific’ (Table 3). The big vein disease rating in all these lines was significantly less than the susceptible control ‘Great Lakes 65’ (Table 3). The downy mildew severity in ‘La Brillante’ was significantly lower than ‘Salinas 88’ (Table 3). The downy mildew severity scores of RH08-0472, and RH08-0475 were lower than ‘Salinas 88’, but the difference was not significant (Table 3).

Availability

Limited seed samples of RH08-0472 and RH08-0475, as well as RH05-0336, RH05-0339, and RH05-0340, are available for distribution to all interested parties for research purposes. These lines are being publically released with no IP protection for the development and commercialization of new cultivars by seed companies, Universities, NGOs or other bonafide private or public research organizations. Samples were deposited in the National Plant Germplasm System as W6 39944

(RH08-0472) and W6 39945 (RH08-0475). It is requested that appropriate recognition be made if the breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to Dr. Ryan Hayes, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905 or sent through email (Ryan.Hayes@ars.usda.gov).

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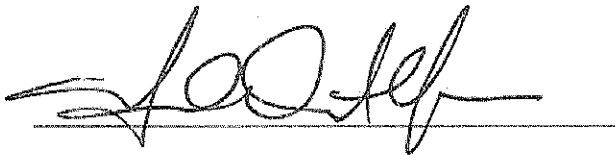
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294 Administrator, Agricultural Research Service
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Table 1. Verticillium wilt disease incidence (DI) in two lettuce iceberg breeding lines and cultivars caused by *Verticillium dahliae* in replicated and unreplicated 2009 and 2010 field experiments in Salinas, CA.

Line	Replicated field experiment artificially infested with race 1 ^z			Unreplicated experiment naturally infested grower field	
	Median	Maximum	RE ^y	Trial 1	Trial 2
	DI	DI		DI	DI
La Brillante	0.0	0.3	0.29 a	0.0	0.0
Salinas 88	0.6	1.0	0.82 b	0.8	1.0
Tiber	0.7	1.0	0.79 b		
RH08-0472	0.1	0.6	0.37 a	0.0	0.0
RH08-0475	0.0	0.4	0.31 a	0.0	0.0

^z/ Results from four experiments. Field was infested with race 1 isolate VdLs16.

^y/ Relative effect calculated from rank analysis of percent infected data. RE followed by different letters indicate significant differences.

Table 2. Horticultural characteristics, yield, percent tipburn, and post harvest quality of lettuce breeding lines and cultivars in unreplicated *Verticillium dahliae* infested field experiments in Salinas, CA and replicated non-infested field experiments in Soledad, CA and Salinas, CA conducted between 2008 and 2010.

Horticultural characteristics and yield													
Line	Harvest date	Head			Core Height (cm)	Maturity 1-5 ^z	Percent Harvestable %	Cartons / Acre ^y	Percent tipburn %	Post harvest quality			
		Weight (g)	Height (cm)	Width (cm)						Shelf-life ^x	Whole head arrival quality ^w		
Trial 1: unreplicated <i>V. dahliae</i> infested grower trial, two seed lines on 102 cm wide beds in Salinas, CA													
La Brillante	9.09.10							0					
La Brillante	9.16.10							100					
RH08-0472	9.09.10	653	13.1	16.0	2.6	4.5	75	813	0				
RH08-0472	9.16.10	741	13.1	16.5	7.1	4.5	60	650	10				
RH08-0475	9.09.10	577	12.1	16.3	2.8	3.0	67	726	0				
RH08-0475	9.16.10	889	12.5	17.8	5.3	4.5	84	910	20				
Salinas 88	9.09.10	623	12.3	16.4	2.9	3.0	59	639	0				
Salinas 88	9.16.10	623	12.7	15.6	4.2	4.5	45	488	30				
Trial 2: unreplicated <i>V. dahliae</i> infested grower trial, five seed lines on 204 cm wide beds in Salinas, CA													
La Brillante	10.01.10								0				
La Brillante	10.08.10								0				
RH08-0472	10.01.10	511	11.6	17.1	2.7	2.5	47	636	0				
RH08-0472	10.08.10	666	12.2	16.3	3.4	4.0	34	460	0				
RH08-0475	10.01.10	451	11.4	15.0	2.1	2.0	26	352	0				
RH08-0475	10.08.10	621	12.0	16.2	3.7	4.0	58	785	0				
Salinas 88	10.01.10	526	12.8	15.6	2.8	2.5	43	582	0				
Salinas 88	10.08.10	479	12.6	15.7	3.4	4.0	8	108	0				
Trial 3: replicated non-infested grower trial, two seed lines on 102 cm wide beds in Soledad, CA													
Pacific	4.29.08	574 ^v	13.5	14.7	2.5		86	932	0				
RH07-0341	4.29.08	782 ^b	12.9	14.3	3.0		65	704	0				
Salinas 88	4.29.08	629 ^{ab}	13.1	14.1	3.4		81	878	0				

Tiber	4.29.08	534 ab	13.2	13.1	2.4	67	726	0
Trial 4: replicated non-infested grower trial, two seed lines on 102 cm wide beds in Soledad, CA								
Pacific	4.30.09	398 ab	12.7	13.2	2.2	3.5	82	888
RH08-0472	4.30.09	611 b	13.2	13.0	2.8	3.0	83	899
RH08-0475	4.30.09	498 ab	12.7	11.7	2.9	3.5	70	758
Salinas 88	4.30.09	323 a	11.6	12.5	2.4	3.3	74	802
Tiber	4.30.09	412 ab	11.7	12.5	2.2	3.2	63	683
Trial 5: replicated non-infested USDA trial, two seed lines on 102 cm wide beds in Salinas, CA								
Tiber	7.10.09	826	12.8	15.8	2.8	3.2	82	888
Pacific	7.10.09	818	14.4	16.4	3.0	3.0	78	845
Salinas 88	7.10.09	701	14.0	16.2	3.1	2.8	82	888
RH08-0472	7.10.09	932	12.7	17.7	2.6	2.5	77	834
RH08-0475	7.10.09	827	12.9	16.8	2.7	3.0	77	834
Triple Threat	7.10.09							10.0 b
Trial 6: replicated non-infested USDA trial, two seed lines on 102 cm wide beds in Salinas, CA								
RH08-0472	8.06.10	1060	13.6 a	18.3	5.4	3.2	66 a	715 a
RH08-0475	8.06.10	974	14.6 ab	18.5	6.6	3.0	25 b	271 b
Salinas 88	8.06.10	1065	15.2 b	16.8	5.7	3.5	85 a	921 a
La Brillante	8.06.10							2.5 a
								8.9 b

^z/ Head maturity visually rated as 1 for immature to 5 for overmature.

^y/ Assumes 26,000 plants per acre on 102 cm wide beds and 32,500 plants per acre on 204 cm wide beds.

^x/ The amount of salad tissue decay was visually evaluated on a scale of 1 to 10, in which 1 is no obvious decay, 10 is complete decay.

^w/ Whole heads were visually rated 1 = no obvious decay, 4 is complete decay.

^v/ Means followed by different letters indicate a significant different at $P < 0.05$.

Table 3. Big-vein disease rating in lettuce breeding lines and cultivars in replicated 2008 and 2009 infested field experiments in Soledad, CA and downy mildew severity in two replicated field experiments conducted in 2010 in Salinas, CA.

Line	Big-vein incidence				Downy Mildew ^y	
	2008		2009		Severity	rAUDPC
	Number Tested	Mean DR ^z	Number Tested	Mean DR		
Great Lakes 65	141	0.79 ^p	118	0.60 ^p		
Pacific	158	0.40	129	0.24		
Tiber	44	0.54	120	0.38	2.9	58.6
Salinas 88	114	0.33	124	0.38	4.5	68.6
La Brillante					1.0 ^s	13.6 ^s
RH07-0341	104	0.54				
RH08-0472			42	0.31	3.0	48.6
RH08-0475			43	0.30	2.8	44.3

^z/ DR = disease rating, backtransformed values from analysis of arcsine transformed percentage big-vein symptomatic plants data.

^y/ Downy mildew resistance was assessed in two independent 2010 field experiments located in Salinas, CA using a scale of 0 for no disease to 5 for severe disease. Severity was scored Oct. 27, 2010 in the first field experiment. Relative area under the disease progress curve (rAUDPC) was calculated in a second experiment using four severity assessment dates spaced seven days apart, beginning on Sept. 22, 2010 and ending on Oct. 13, 2010.

^p Significantly higher disease rating than 'Pacific'.

^s Significantly lower than 'Salinas 88'.