

**Santa Catarina State Agricultural Research and Rural Extension Agency
Itajaí Experimental Station**

**Development of rice (*Oryza sativa* L.) lines resistant to
herbicides through seed induced mutation with gamma
rays**

LAERTE REIS TERRES



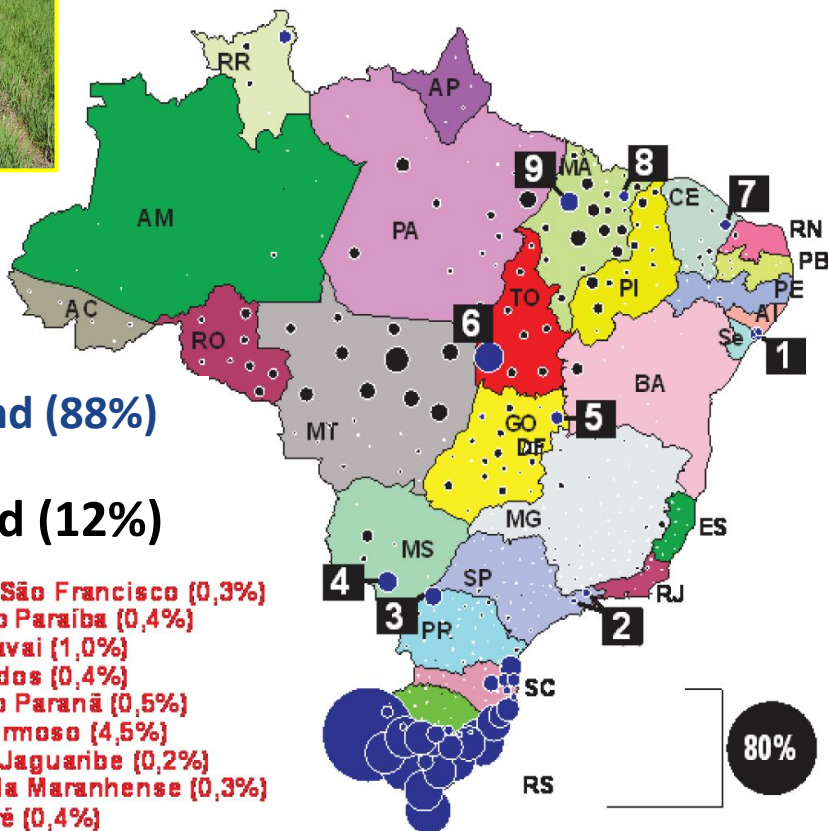
Andrade, A. Dr.
Terres, L.T.
Marschalek, R. Dr
Pereira, A. M.Sc.
Scheuermann, K.K., Dr.
Wickert, E. Dra
Noldin J.A., Dr.

Vienna - 2024



Projeto Arroz

Rice Team



Approx. 1.1 Mi ha

Dry-seeded system (78%)

Pre-germinated system (22%)



Rice Production in Santa Catarina State



Approx. 150 K ha - 94 county

Pre-germinated system (90%)

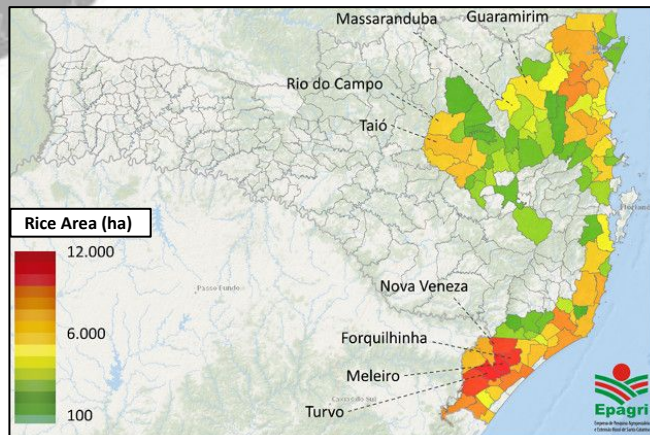
Dry-seeded system (10%)

Approx. 5 k producers involved in rice cultivation

Average cultivated area: 39,7 ha

Average Yield 8200 Kg/ha in the last 4 years

27 processing industries



Rice in Santa Catarina

- 150.000 ha
- 6.000 families
- 83 counties (growing rice in Santa Catarina)
- 1.050 mil t / Production
- 8,0 t/ha Yield
- Yields of 10 t/ha are very common among farmers that use Epagri's recommended technologies





Banco germoplasma

Mutantes

Hibridação

Gerações segregantes

Mudas



Melhoramento genético Arroz Irrigado

Apoio:



Exp. Regional

Seleção

Avaliação preliminar

Análise sensorial

NOVAS CULTIVARES



Rice Breeding

- Cultivar development
- Adapted to local conditions
- Yield
- Disease tolerance
- Abiotic stress tolerance
- Grain quality



Rice breeding programme

- Pedigree Method
- Until 2024 Epagri released 33 varieties (26 for Santa Catarina)





EPAGRI RICE CULTIVARS 1980-2023



Mutant Variety

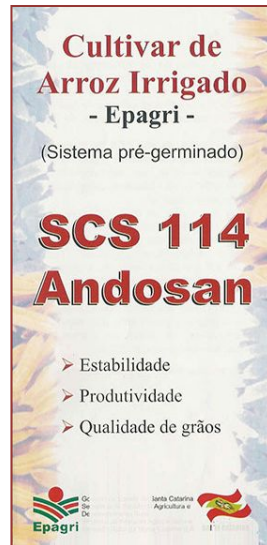
Rice Mutant Cultivar SCS114 Andosan

2005

T. Ishiy¹, M.S. Schiocchet¹, R.E. Bacha¹, D. Alfonso-Morel¹, A. Tulman Neto² and R. Knoblauch¹

¹Epagri/Estação Experimental de Itajaí. Cx. 277, 88351-970, Itajaí, SC, Brazil, e-mail: mschio@epagri.rct-sc.br

²Centro de Energia Nuclear na Agricultura. Cx. 96, 13400-970, Piracicaba, SP, Brazil, e-mail: tulmann@cena.usp.br



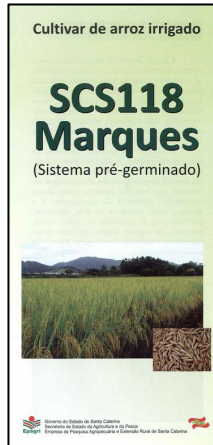
CULTIVAR RELEASE

SCS118 Marques – New rice cultivar obtained through induced mutation

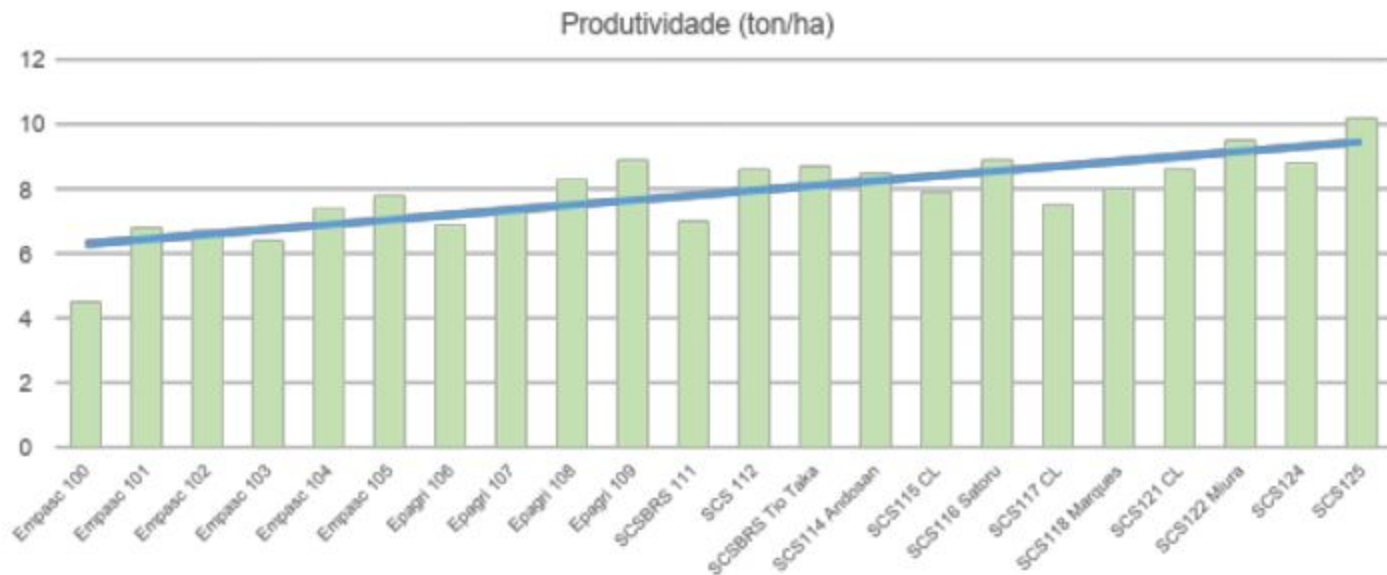
2013

Moacir Antonio Schiocchet^{1*}, Jose Alberto Noldin¹, Juliana Vieira Raimondi², Augusto Tulmann Neto³, Rubens Marschalek¹, Ester Wickert¹, Gabriela Neves Martins¹, Eduardo Hickel¹, Ronaldir Knoblauch¹, Klaus Konrad Scheuermann¹, Domingos Savio Eberhardt¹ and Alexander De Andrade¹

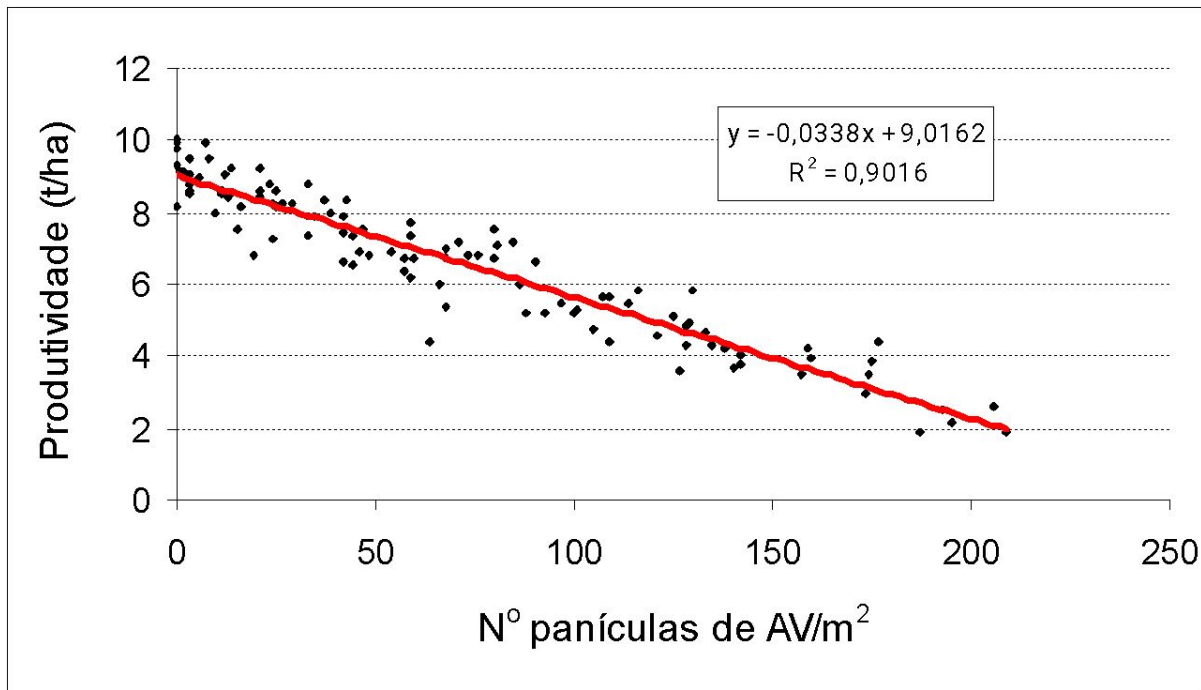
Received 20 February 2013



GENETIC GAIN



□ Weed rice causes losses to rice yield (Itajaí, SC)



□ Yield losses of **34 kg/ha** for each weed rice panicle/m²

Con herbicida

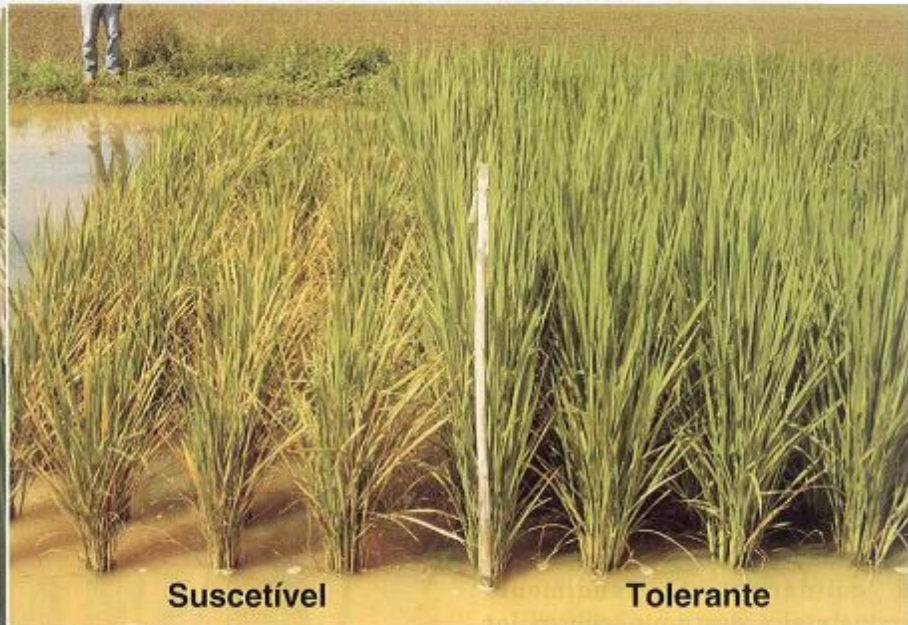


Sin herbicida





SCS 115 CL: primeira cultivar de arroz irrigado para uso no sistema Clearfield de produção



SCS117 CL: NOVA CULTIVAR DE ARROZ IRRIGADO

Moacir Antonio Schiocchet¹; Rubens Marschalek²; José Alberto Noldin³; Juliana Vieira Raimondi⁴
Domingos Sávio Eberhardt⁵; Gabriela Neves Martins⁶; Richard Elias Bacha⁷,



CULTIVAR RELEASE

<http://dx.doi.org/10.1590/1984-70332015v15n4c47>

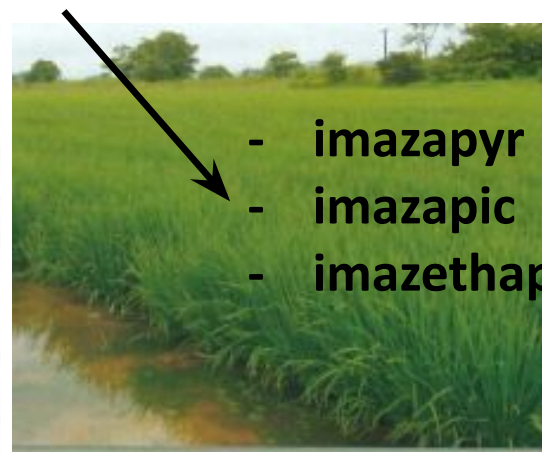
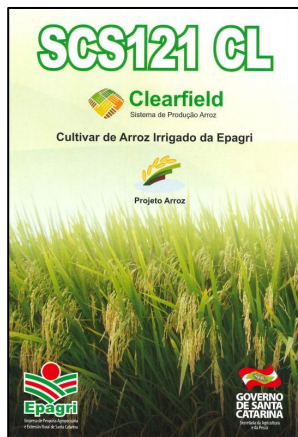
SCS121 CL: Rice cultivar resistant to herbicides of imidazolinone chemical group

Moacir Antonio Schiocchet¹, Jose Alberto Noldin¹, Rubens Marschalek¹, Ester Wickert¹, Gabriela Neves Martins¹, Domingos Savio Eberhardt¹, Eduardo Hickel¹, Ronaldir Knoblauch¹, Klaus Konrad Scheuermann¹, Juliana Vieira Raimondi² and Alexander de Andrade^{1*}



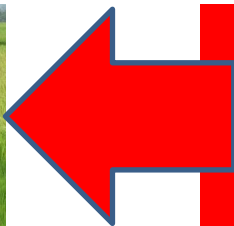
Inhibit the enzyme
acetohydroxyacid synthase
(AHAS), also called
acetolactate synthase (**ALS**)

Several variant ALS genes conferring imidazolinone tolerance were discovered in plants through mutagenesis and selection



- imazapyr
- imazapic
- imazethapyr

CLEARFIELD (CL) Rice Production System



SCS116 Satoru

(not resistant to ALS (IMI) herbicides)

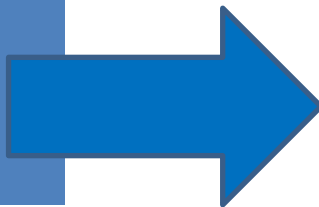
(BASF/Epagri)



CLEARFIELD®
production system |

SCS121 CL

Resistant to ALS (IMI) Herbicides



Proud of it / concern about it...



- 2021 - About **60%** of the rice area in SC was sowed by one single Epagri clearfield variety (SCS121 CL)
- What about food security - weed resistance to herbicides - the risk of diseases (blast) etc?

- Gene flow are causing weed rice resistance to “ALS inhibitor herbicides” (IMI herbicides)
- Weed selection through herbicide selection

Epagri's Rice Team started to look for new herbicide resistance traits through mutagenesis (up to 2010)

Mutation in Rice at Epagri

Aims:

Grain quality, yield, herbicide tolerance, reducing height

- Gamma rays mutagenesis:
0,25 - 0,35 kGy
- Chemical mutagenesis:
EMS – Ethyl methanesuphonate (1%)
Sodium azide (0.0025M)







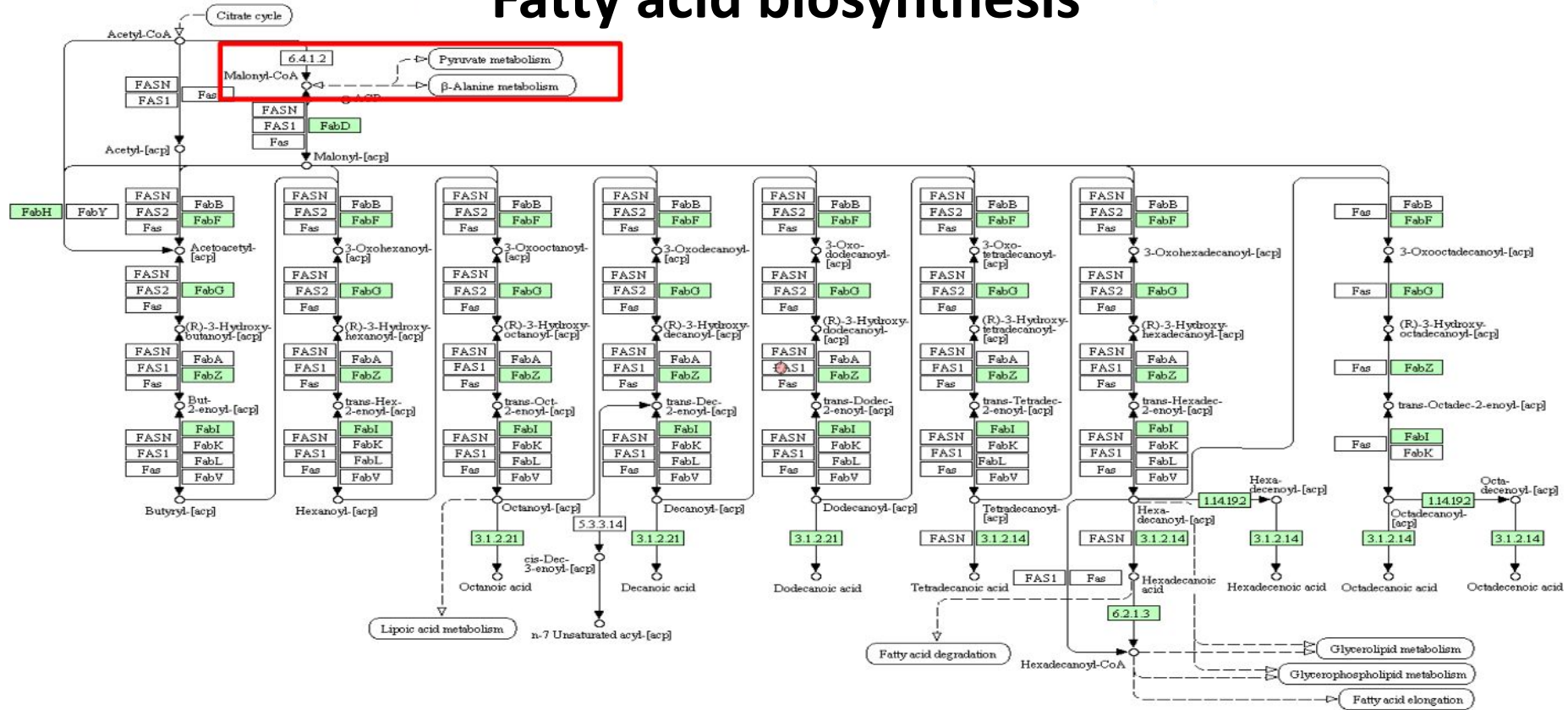




- Selection through Herbicides inhibiting acetyl-coenzyme A carboxylase (ACCase)
- ACCase inhibitors block fatty acid biosynthesis resulting in plant dead.
- Resistance is conferred by a single point mutation with an amino acid substitution of the carboxyl transferase domains of the ACCase gene



Fatty acid biosynthesis



Seeds of Epagri's rice variety 'Sabbore' were irradiated

Prof. Dr.

Augusto Tulmann Neto

(USP – SP - Brazil)

200-300 Gy Gamma Rays

Center of Nuclear Energy
for Agriculture - USP



1. M1 seeds sowed
2. From the M1 plants 8-10 Seeds per plant were used to generate the M2 population
3. The 200.000 plants M2 population were screened for the herbicide resistance about 15 days after emergency (V3-V4
4. Herbicide: Quizalofop-p-ethyl 60g a.i./ha
5. Survived plants were transplanted into a greenhouse
6. The resulting M3 progenies were tested again under quizalofop to confirm the resistance
7. 2 progenies were selected
8. The 2 M4 resistant pop. show no herbicide injury (R lines)



Resistant plants through Epagri's "ACCCase System"


Received: 15 December 2017 | Accepted: 12 March 2018

DOI: 10.1111/pbr.12592

ORIGINAL ARTICLE

WILEY  Plant Breeding

Development of rice (*Oryza sativa*) lines resistant to aryloxyphenoxypropionate herbicides through induced mutation with gamma rays

Alexander de Andrade¹  | Augusto Tulmann-Neto² | Fernando A. Tcacenco³ |
Rubens Marschalek¹ | Adriana Pereira¹ | Antonio M. de Oliveira Neto⁴ |
Klaus K. Scheuermann¹ | Ester Wickert¹ | José A. Noldin¹

Resistance evaluation of Epagri's "ACCase system"

Quizalofop (TARGA*)
Sabbore variety (not
resistant to the
herbicide)

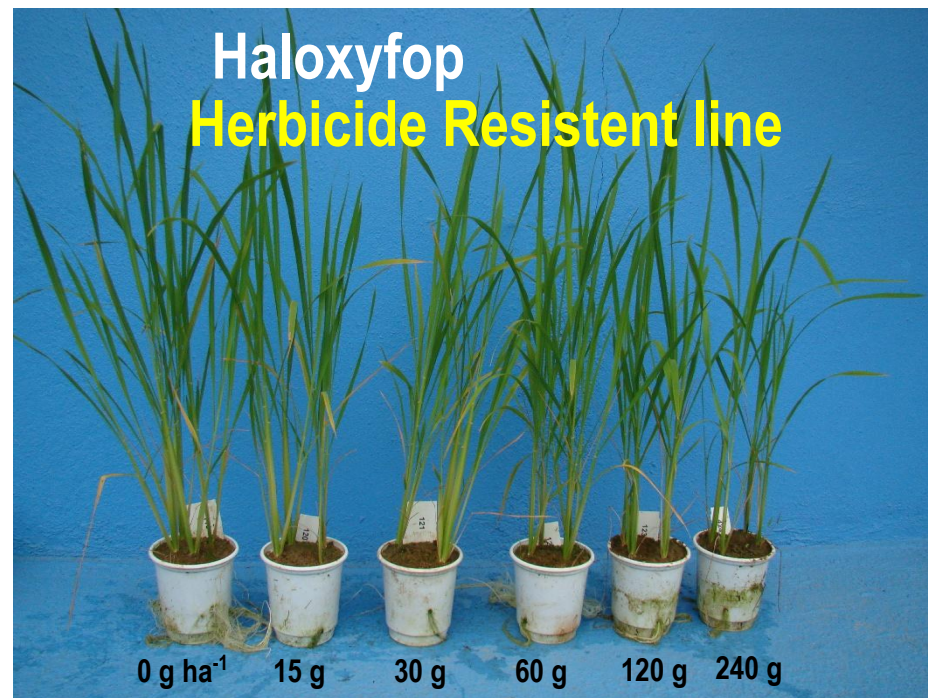
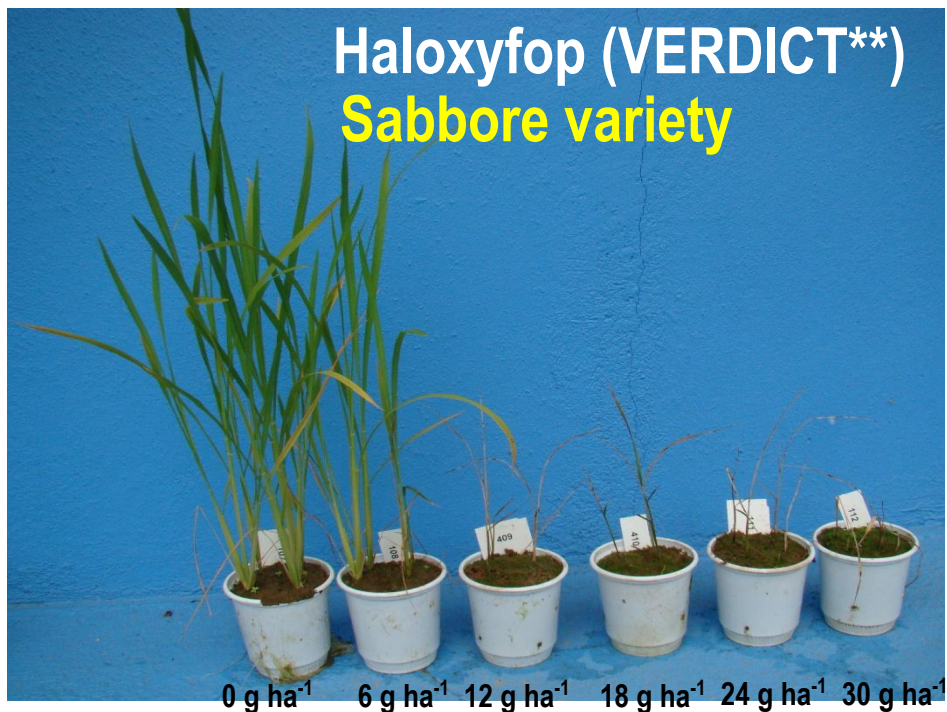
0 g ha⁻¹ 7,5 g ha⁻¹ 15 g ha⁻¹ 22,5 g ha⁻¹ 30 g ha⁻¹ 37,5 g ha⁻¹

Quizalofop (TARGA)
Resistant line

0 g ha⁻¹ 18,75 g 37,5 g 75 g ha⁻¹ 150 g 300 g

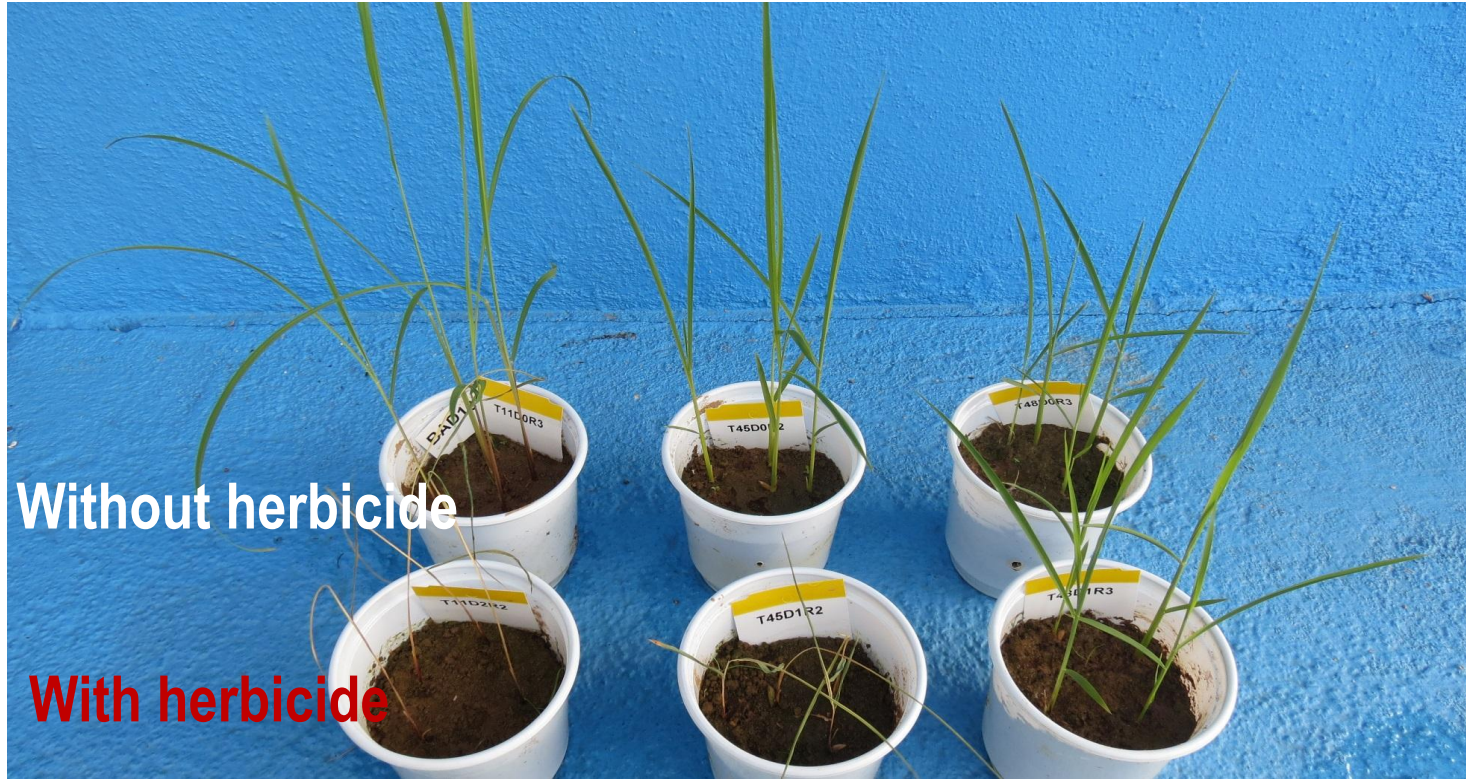
* BASF

Evaluation of resistance of Epagri's ACCase system



** Corteva Agriscience

Rice resistant to ACCase inhibitors herbicides



weed-rice

SCS121 CL

Epagri resistant line

- DNA from R and S lines were taken
- Eight set of primer were generated/generated based on the chloroplastic ACCase sequences
- The amplified DNA was sequenced
- Sequencing of the carboxyl-transferase region ACCase gene revealed one single pair change (transversion G>T) that was found in the coding region of the ACCase gene at the position 2027.
- This caused an amino acid change in the ACCase protein: from tryptophan to cysteine.

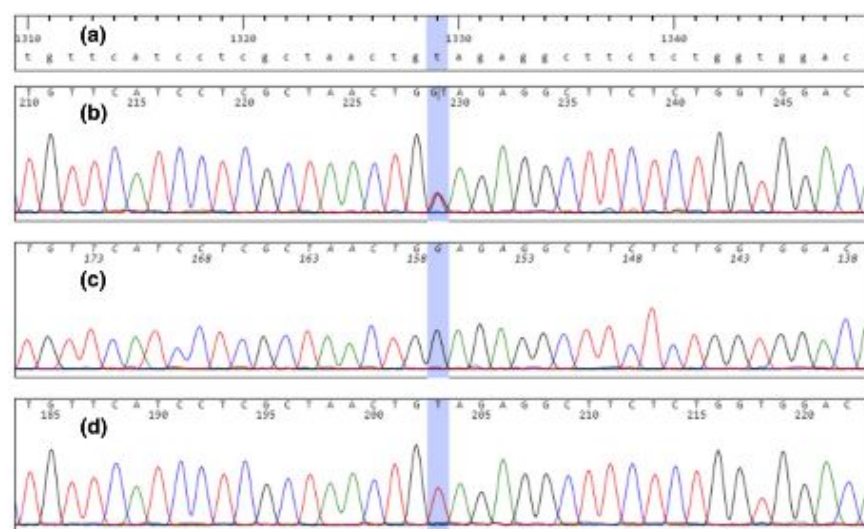
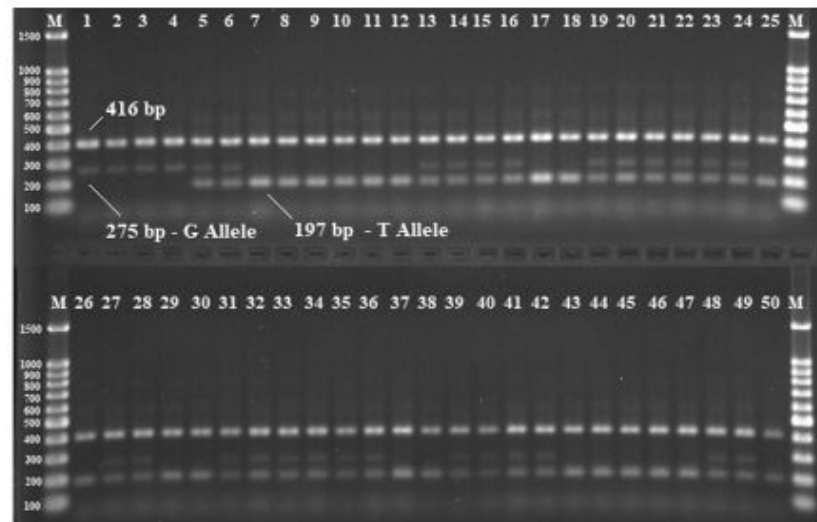
Carboxyl-transferase sequence site of Acetyl coenzima A carboxylase (ACCase) gene

A.	FONTE	SEQUÊNCIA (numeração correspondente ao gene ACCase de <i>Alopecurus myosuroides</i> , <u>GenBank locus</u> CAC84161.1, <u>EMBL</u> accession AJ310767.1/mostrada parcialmente)
701- 800	GENBANK US/2013 EPAGRT	<p>T.AANSGARTGTADFEVKSCEFRVGSDDGSPERGFEOYIYT.SSEEDYARIGTSVTAHKMOL.DSGETRHWVTDVSVGKEDGT.GVFNTHGSAATASAY</p> <p>T.AANSGARTGTADFEVKSCEFRVGSDDGSPERGFEOYIYT.SSEEDYARIGTSVTAHKMOL.DSGETRHWVTDVSVGKEDGT.GVFNTHGSAATASAY</p> <p>T.AANSGARTGTADFEVKSCEFRVGSDDGSPERGFEOYIYT.SSEEDYARIGTSVTAHKMOL.DSGETRHWVTDVSVGKEDGT.GVFNTHGSAATASAY</p>
801- 900	GENBANK US/2013 EPAGRT	<p>L.TFVVTGRTVGTGAYLART.GTRCTORT.DOPTIITGYSATLNKLLGREVYSSHMOL.GGPKIMATNGVVHITVSDDLFGVSNITL.RWT.SVVPAYTG</p> <p>L.TFVVTGRTVGTGAYLART.GTRCTORT.DOPTIITGYSATLNKLLGREVYSSHMOL.GGPKIMATNGVVHITVSDDLFGVSNITL.RWT.SVVPAYTG</p> <p>L.TFVVTGRTVGTGAYLART.GTRCTORT.DOPTIITGYSATLNKLLGREVYSSHMOL.GGPKIMATNGVVHITVSDDLFGVSNITL.RWT.SVVPAYTG</p>
901- 000	GENBANK US/2013 EPAGRT	<p>DPPDRPVAYIPENSCDPRAAIRGVDDSGKWLGGMFDDKDSFVETFEGWAKTVVVTGRAKLGGIPVGVIAVETOTMMOTIPADPGOLDSREOS</p> <p>DPPDRPVAYIPENSCDPRAAIRGVDDSGKWLGGMFDDKDSFVETFEGWAKTVVVTGRAKLGGIPVGVIAVETOTMMOTIPADPGOLDSREOS</p> <p>DPPDRPVAYIPENSCDPRAAIRGVDDSGKWLGGMFDDKDSFVETFEGWAKTVVVTGRAKLGGIPVGVIAVETOTMMOTIPADPGOLDSREOS</p>
001- 100	GENBANK US/2013 EPAGRT	<p>PDSATKTAQATIT.DFNREGT.PI.FITLANWRGFSGGORDI.FEGTII.OAGSTIVENT.RTYNOPA.FVYTPMAAET.RGGAWVVVDSKINPDRTFCYAE</p> <p>PDSATKTAQATIT.DFNREGT.PI.FITLANWRGFSGGORDI.FEGTII.OAGSTIVENT.RTYNOPA.FVYTPMAAET.RGGAWVVVDSKINPDRTFCYAE</p> <p>PDSATKTAQATIT.DFNREGT.PI.FITLANWRGFSGGORDI.FEGTII.OAGSTIVENT.RTYNOPA.FVYTPMAAET.RGGAWVVVDSKINPDRTFCYAE</p>
101- 200	GENBANK US/2013 EPAGRT	<p>POGLTETKFRSRET.ODCMSRT.DPTIITDKAKLEEVANKNGSADTST.OENTFARTKOLMPLYTOTATREFART.HDTST.RMAAKGVTEKKVVDWE</p> <p>POGLTETKFRSRET.ODCMSRT.DPTIITDKAKLEEVANKNGSADTST.OENTFARTKOLMPLYTOTATREFART.HDTST.RMAAKGVTEKKVVDWE</p> <p>POGLTETKFRSRET.ODCMSRT.DPTIITDKAKLEEVANKNGSADTST.OENTFARTKOLMPLYTOTATREFART.HDTST.RMAAKGVTEKKVVDWE</p>
201- 300	GENBANK US/2013 EPAGRT	<p>LRRRISEDVLAKEIRAVAGEOFSHOPAIELIKKWSASASHAAEWDDDDAFVAMMDNPNENYKDYIOYLKAORVSOSLSLSDSSSDLOALPOG</p> <p>LRRRISEDVLAKEIRAVAGEOFSHOPAIELIKKWSASASHAAEWDDDDAFVAMMDNPNENYKDYIOYLKAORVSOSLSLSDSSSDLOALPOG</p> <p>LRRRISEDVLAKEIRAVAGEOFSHOPAIELIKKWSASASHAAEWDDDDAFVAMMDNPNENYKDYIOYLKAORVSOSLSLSDSSSDLOALPOG</p>



Detecting acetyl-coenzyme a carboxylase resistance gene in rice (*Oryza sativa* L.)

Adriana Pereira¹ · Fernando Adami Tcacenco² · Gustavo Henrique Ferrero Klabunde¹ · Alexander de Andrade¹



16/07/2015 860.150.150787
10:54 NPWB
0000221505309918

BR 10 2015 017012 2



Protocolo

Número

Código QR



INPI
INSTITUTO
NACIONAL
DA PROPRIEDADE
INDUSTRIAL

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

Diretoria de Patentes
Sistema e Patentes/Depósito

DIRPA **PATENTES**

Tipo de Documento:

Recibo de Peticionamento Eletrônico

DIRPA

Página:
1 / 2

Título do Documento:

Recibo

DIRPA-FQ001 - Depósito de Pedido de Patente ou de Certificado de Adição

Código:
RECIBO

Versão:
01

Modo:
Produção

O Instituto Nacional da Propriedade Industrial informa:

Este é um documento acusando o recebimento de sua petição conforme especificado abaixo:

Dados do INPI:

Número de processo: BR 10 2015 017012 2
Número da GRU principal: 00.000.2.2.15.0530991.8 (serviço 200)
Número do protocolo: 860.150.150787
Data do protocolo: 16 de Julho de 2015, 10:54 (BRT)
Número de referência do envio: 121648

Dados do requerente ou interessado:

Tipo de formulário enviado: DIRPA-FQ001 v.006
Referência interna: P842Epagri
Primeiro requerente ou interessado: Epagri - Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina
CNPJ do primeiro requerente ou interessado: 83.052.191/0001-62
Número de requerentes ou interessados: 1
Título do pedido: Desenvolvimento de linhagens de arroz (*Oryza sativa* L.) com resistência a herbicidas inibidores da enzima acetyl coenzima A carboxilase (ACCase) obtidas por mutação induzida com raios gama

Patent

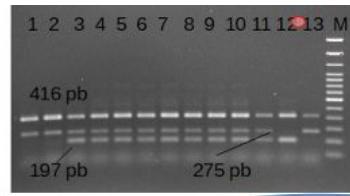
Yield kg/ha

Nowadays:

	SC 965	SC 964
Tratamentos	Média	Média
0,75L/ha Targa	8217,55	8234,304
1,5L/ha Targa	8130,57	7477,075
3,0L/ha Targa	8000,64	8169,742
4,5L/ha Targa	8165,99	7881,681
6,0 L/ha Targa	6660,72	7779,142
0,5 L/ha Verdict	8214,98	8247,369
1,0 L/ha Verdict	8757,16	7816,307
2,0 L/ha Verdict	8235,79	7781,806
4,0 L/ha Verdict	7286,35	6837,216
Control	7361,57	7211,576



Reacciones de PCR
24 marcadores SSR

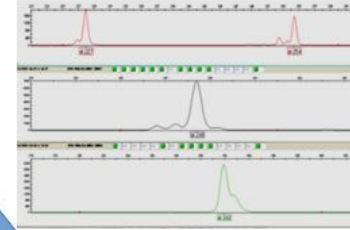


Electroforesis horizontal



Electroforesis capilar

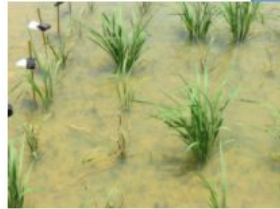
Genotipagem



Electroferograma



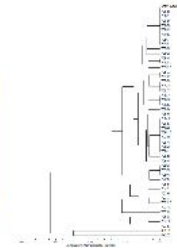
Extracción de ADN



Selecion Herbicida



BACKCROSS



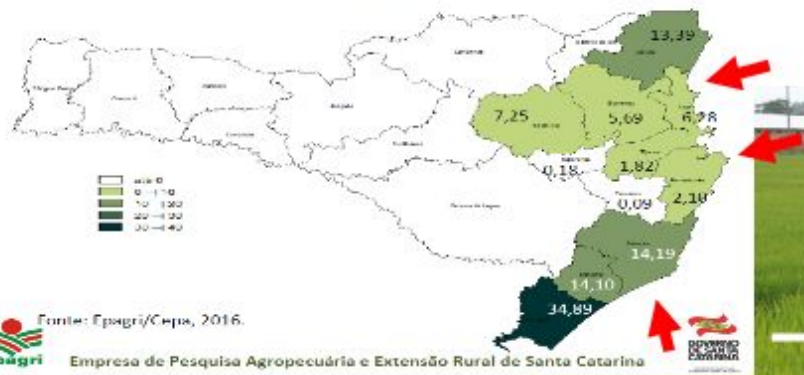
Dendograma

Nowadays:

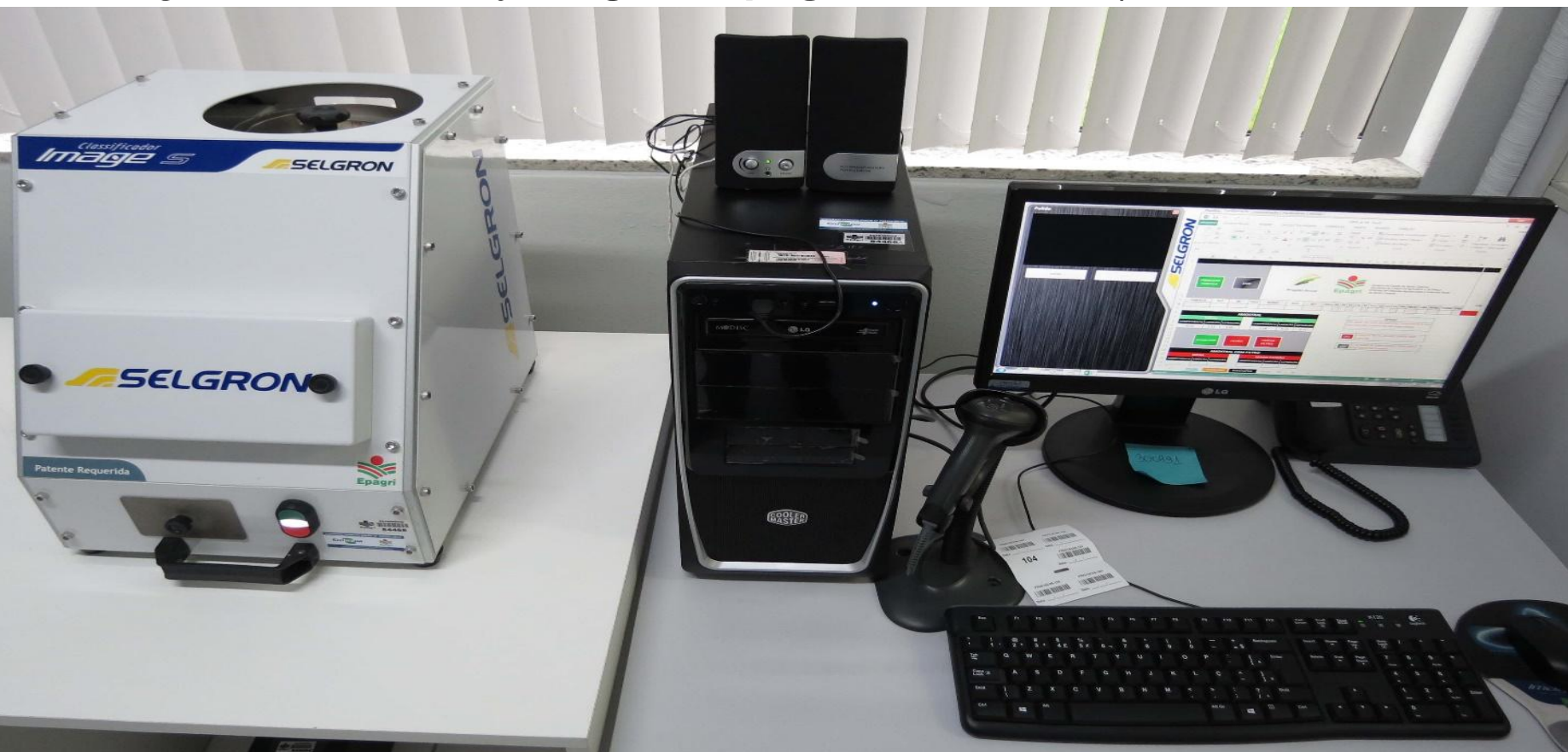


LARGE TRIAL

Distribuição % da área de arroz por microrregiões de SC –
Safrá 2015/16



Grain quality at **Image Rice Grain Scanner** (digital platform for breeding and research by Selgron/Epagri, CBAB 2017)



40 grain quality traits for each sample in 3 minutes

three-dimensional measurement of grain size, dimensional classes, number of chalked grains and chalked area, defects of the grain, milling quality, length/width ratio, % whole grains, % broken grains, etc

Results in a *Microsoft excel spreadsheet*

<http://www.sbmp.org.br/cbab/siscbab/uploads/c8eb9792-c9e1-ce1f.pdf>

SOFTWARE/DEVICE RELEASE

Image - Rice Grain Scanner: a three-dimensional fully automated assessment of grain size and quality traits

Rubens Marschalek¹, Mauricio Cesar Silva¹, Samuel Batista dos Santos¹, Johnny Ricardo Manke², Carlos Biegging², Geovani Porto¹, Ester Wickert¹ and Alexander de Andrade¹

Abstract: The Image is a scanner developed as a grain classifier for quality control at the rice industry based on Brazilian official norms. It orders the dehulled grains ensuring that each grain would pass individually, in free fall, while the grain is analysed from different sides, covering its whole surface. It ensures a precise three-dimensional measurement of grain size, chalkiness, defects of the grain, milling quality, given out a total of 39 traits/classes/defects/values, which



Crop Breeding and Applied Biotechnology

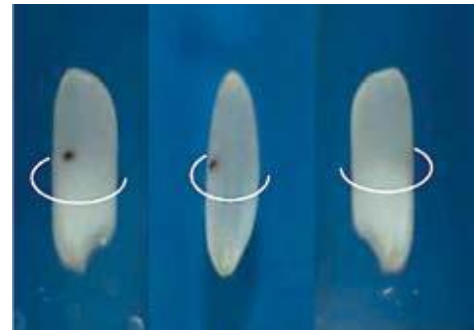
17: 89-97, 2017

Brazilian Society of Plant Breeding,

Printed in Brazil

[http://dx.doi.org/10.1590/1984-](http://dx.doi.org/10.1590/1984-70332017v17n1s15)

70332017v17n1s15



Grain quality by Image Rice Grain Scanner (Selgtron)

Parcela	Comprimento do grão	Largura Grão	Espessura grão	Relação compr/largura	PESO TOTAL DA AMOSTRA	Bom %	Bom + Barriga Branca %	Picados ou Manchados %	Área Gessada % (Centro Branco Oficial Brasil)	Grãos não gessados %	Grãos gessados %	Amarelos e Destoante %	Longo Fino %	Longo %	Médio %	Curto %	Inteiro %	Quebrados %	Inteiro %	Quebrado %	Renda %	Means (kg.ha)
RH2017CV1-1	7,13	2,00	1,70	3,56	31,13	86,61	86,61	6,54	0,97	98,40	1,60	4,43	19,87	68,35	2,76	1,23	92,64	6,51	57,64	4,10	61,74	7335
RH2017CV1-2	7,02	2,01	1,70	3,50	29,25	88,44	88,44	5,49	1,08	98,09	1,91	3,70	17,47	67,18	3,19	1,26	89,58	9,55	52,38	5,60	57,99	6942
RH2017CV1-4	6,99	2,05	1,73	3,41	35,46	88,15	88,15	5,21	1,44	97,78	2,22	3,64	10,66	79,39	2,99	0,61	93,97	5,31	66,63	3,76	70,40	6794
RH2017CV1-6	6,93	2,12	1,72	3,27	34,91	89,58	89,58	3,28	2,01	96,15	3,85	3,87	5,38	82,90	4,51	0,38	94,03	5,23	65,65	3,66	69,31	8053
RH2017CV1-7	7,04	2,14	1,75	3,29	36,31	90,36	90,36	2,71	2,30	96,76	3,24	3,19	4,02	85,59	2,60	0,37	93,81	5,43	68,10	3,97	72,07	10273
RH2017CV1-8	7,09	2,16	1,75	3,29	35,49	91,06	91,06	2,29	2,35	96,67	3,33	2,95	2,38	86,39	2,52	0,47	93,04	6,15	66,03	4,37	70,40	10369
RH2017CV1-10	7,32	2,07	1,69	3,54	33,74	89,66	89,66	4,23	1,38	97,50	2,50	3,23	8,52	80,89	1,64	0,92	92,77	6,41	62,65	4,29	66,94	9726
SC 964	6,85	2,17	1,79	3,16	37,93	92,98	92,98	2,88	0,54	98,88	1,12	2,44	2,11	88,62	4,36	0,21	96,27	3,01	73,01	2,29	75,30	8897
SC 965	6,81	2,16	1,78	3,15	36,86	94,95	94,95	1,65	0,67	98,67	1,33	1,74	2,23	85,14	6,98	0,25	95,67	3,59	70,53	2,65	73,18	8839
SCS121 CL	7,19	2,19	1,77	3,28	30,76	80,22	80,22	13,07	0,94	97,93	2,07	3,59	2,17	80,08	1,56	1,00	88,00	11,03	54,21	6,71	60,92	10893

Grain quality analysis:

- *Sensory tests
- *Cooking quality
- *Amylose content

URBANO
Como faz bem.

Embrapa
Arroz e Feijão



- Still using Gamma ray irradiation to induce mutation for:
 - Herbicide resistance
 - Agronomic traits
 - Tolerance to abiotic stress



Conclusions:

- Gamma ray irradiation was able to create new rice mutants with tolerance to Haloxy/Quizalofop's herbicide.
- It will take 2 years more to release a new "ACCase system" resistant cultivar with good yields and grain quality.
- "ACCase system" varieties would help to manage weeds and alternate herbicides and varieties of the Clearfield (CL) system.
- Gamma ray has a great potential in rice breeding.





PARTNERS

