

The Rice example: illustrating the first five steps for smoothing and extracting traits (SET) using growthPheno

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This example is based on the data whose analysis has been published by Al-Tamimi et al. (2016). The five steps of the method for smoothing and extracting traits (SET) described in detail in Brien et al. (2020) is illustrated for this data using `growthPheno` (Brien, 2026g), a package for the R statistical computing environment (R Core Team, 2026).

Initialize

Step 1: Import, select and derive longitudinal data

Step 1(a): Import the data

```
data(RiceRaw.dat)
```

Step 1(b): Organize the data

Here the imaging variables are selected and covariates and factors added to produce `longi.dat`.

```
longi.dat <- prepImageData(data=RiceRaw.dat,
                           potIDcolumns = c("Genotype.ID", "Treatment.1", "Replicate" ),
                           smarthouse.lev=c("NE", "NW"))

longi.dat <- designFactors(data = longi.dat, insertName = "Replicate",
                           nzones = 3, designfactorMethod="StandardOrder")

### Particular edits to longi.dat - add Days after treatment (xDAT)
longi.dat$xDAT <- longi.dat$xDAP - 29
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag,DAP), ])
```

Step 1(c): Derive longitudinal traits that result in a value for each observation

```

# Set responses
responses.image <- c("PSA")
responses.smooth <- paste0("s", responses.image)

# Form growth rates for each observation of a subset of responses by differencing
longi.dat <- byIndv4Times_GRsDiff(longi.dat, responses = responses.image,
                                times = "DAP",
                                which.rates = c("AGR", "RGR"))

# Form PSA.WUI
longi.dat <- within(longi.dat,
                    PSA.WUI <- WUI(PSA.AGR*DAP.diffs, WU))

# Add cumulative responses
longi.dat <- within(longi.dat,
{
  WU.cum <- unlist(by(WU, Snapshot.ID.Tag,
                     cumulate, exclude.1st=TRUE))
  WUI.cum <- PSA / WU.cum
})

# Check longi.dat
head(longi.dat)

```

```

##   Smarthouse Lane Position DAP xDAP Snapshot.ID.Tag Snapshot.Time.Stamp
## 1      NE      1          2  28  28      045451-C 2015-02-18 02:14:00
## 2      NE      1          2  30  30      045451-C 2015-02-20 02:14:00
## 3      NE      1          2  31  31      045451-C 2015-02-21 02:14:00
## 4      NE      1          2  32  32      045451-C 2015-02-22 02:14:00
## 5      NE      1          2  33  33      045451-C 2015-02-23 02:14:00
## 6      NE      1          2  34  34      045451-C 2015-02-24 02:14:00
##   Hour Genotype.ID Treatment.1 Replicate Zone cZone SHZone ZLane ZMainunit
## 1 2.233333      121080   Control      1      1      -1      1      1      1
## 2 2.233333      121080   Control      1      1      -1      1      1      1
## 3 2.233333      121080   Control      1      1      -1      1      1      1
## 4 2.233333      121080   Control      1      1      -1      1      1      1
## 5 2.233333      121080   Control      1      1      -1      1      1      1
## 6 2.233333      121080   Control      1      1      -1      1      1      1
##   Subunit cMainPosn cPosn Weight.Before Weight.After Water.Amount WU PSA
## 1      1      -10.5  -11      4007      4031      28 NA 57.446
## 2      1      -10.5  -11      4056      4084      32 -25 89.306
## 3      1      -10.5  -11      4036      4083      52 48 100.138
## 4      1      -10.5  -11      4027      4085      61 56 128.323
## 5      1      -10.5  -11      4019      4084      69 66 158.776
## 6      1      -10.5  -11      4014      4083      74 70 182.551
##   PSA.SV1 PSA.SV2 PSA.TV Boundary.Points.To.PSA.Ratio.SV1
## 1 20.912 11.526 25.008      0.353912
## 2 29.073 21.495 38.738      0.310735
## 3 27.751 26.835 45.552      0.354293
## 4 34.697 32.848 60.778      0.371012
## 5 46.779 37.871 74.126      0.319823
## 6 48.849 48.794 84.908      0.328400
##   Boundary.Points.To.PSA.Ratio.SV2 Boundary.Points.To.PSA.Ratio.TV
## 1      0.454104      0.197537

```

```

## 2          0.401396          0.172182
## 3          0.332364          0.174175
## 4          0.358469          0.178157
## 5          0.347179          0.172517
## 6          0.290220          0.163153
## Caliper.Length.SV1 Caliper.Length.SV2 Caliper.Length.TV Compactness.SV1
## 1          666.013          668.692          704.189          0.0930821
## 2          632.735          729.044          830.812          0.1327200
## 3          731.077          931.028          1104.350          0.0925419
## 4          791.760          878.427          1029.300          0.0969068
## 5          830.360          965.221          1197.530          0.1241550
## 6          1103.050          991.259          1408.310          0.0938637
## Compactness.SV2 Compactness.TV Convex.Hull.PSA.SV1 Convex.Hull.PSA.SV2
## 1          0.0689923          0.1435880          224.662          167.062
## 2          0.0734412          0.1091450          219.055          292.683
## 3          0.0678337          0.0950009          299.875          395.600
## 4          0.0707469          0.1102850          358.045          464.303
## 5          0.0783589          0.1119250          376.780          483.302
## 6          0.1014870          0.0947390          520.425          480.792
## Convex.Hull.PSA.TV Center.Of.Mass.Y.SV1 Center.Of.Mass.Y.SV2
## 1          174.165          1841.78          1788.86
## 2          354.921          1837.62          1797.42
## 3          479.490          1826.88          1757.60
## 4          551.097          1798.03          1750.54
## 5          662.283          1796.70          1781.50
## 6          896.231          1809.42          1778.94
## Max.Dist.Above.Horizon.Line.SV1 Max.Dist.Above.Horizon.Line.SV2 xDAT
## 1          620          624 -1
## 2          543          541 1
## 3          642          633 2
## 4          736          823 3
## 5          658          652 4
## 6          639          633 5
## DAP.diffs PSA.AGR PSA.RGR PSA.WUI WUI.cum WU.cum
## 1          NA          NA          NA          NA          NA          NA
## 2          2 15.930 0.2206116 -1.2744000 -3.5722400 -25
## 3          1 10.832 0.1144806 0.2256667 4.3538261 23
## 4          1 28.185 0.2480013 0.5033036 1.6243418 79
## 5          1 30.453 0.2129439 0.4614091 1.0950069 145
## 6          1 23.775 0.1395352 0.3396429 0.8490744 215

```

Step 2: Exploratory analysis

Step 2(a): Fit splines to smooth the longitudinal trends in the primary traits and calculate their growth rates

The `smoothing.method` used is `direct` and `df` is set to 4. The growth rates are calculated by difference, rather than from the spline derivatives.

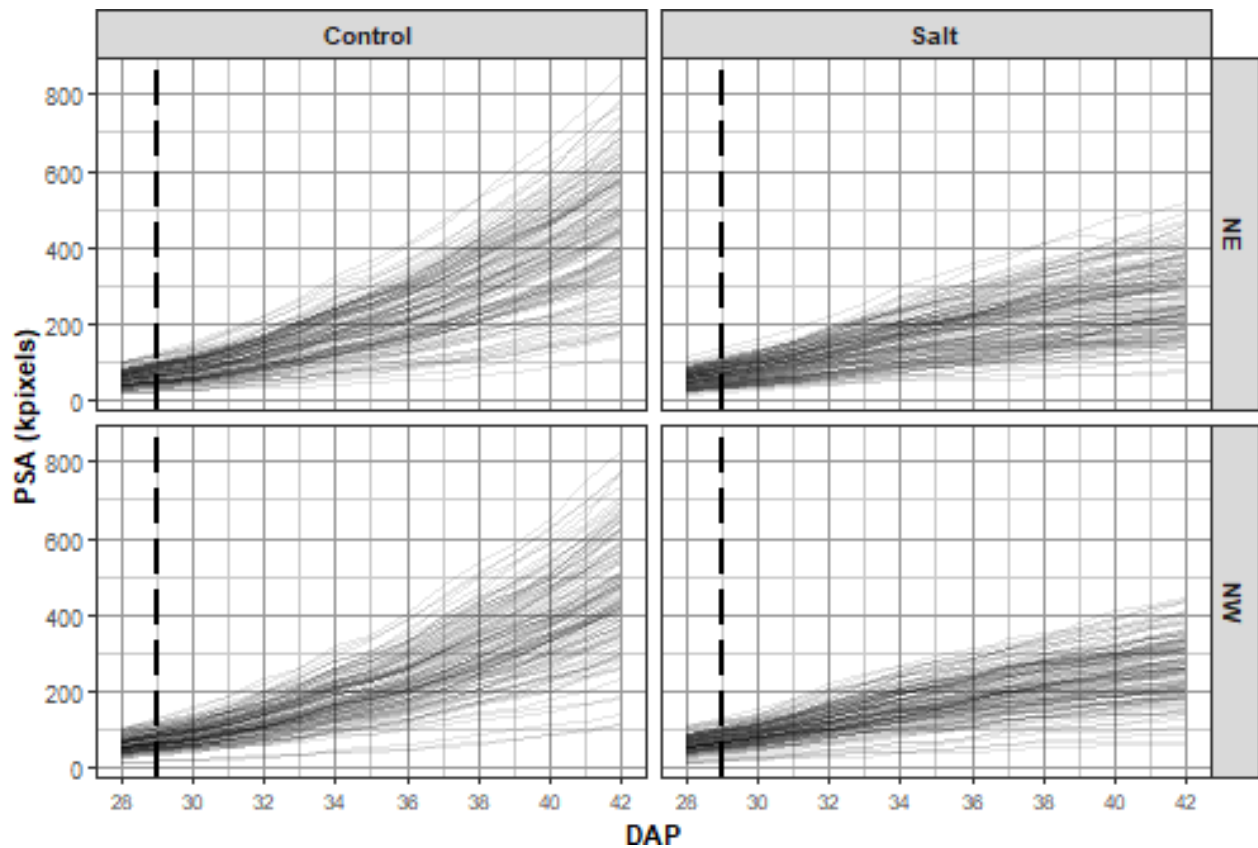
```

# Smooth responses and form growth rates by differences
for (response in c(responses.image, "WU"))
  longi.dat <- byIndv4Times_SplinesGRs(data = longi.dat, response = response,

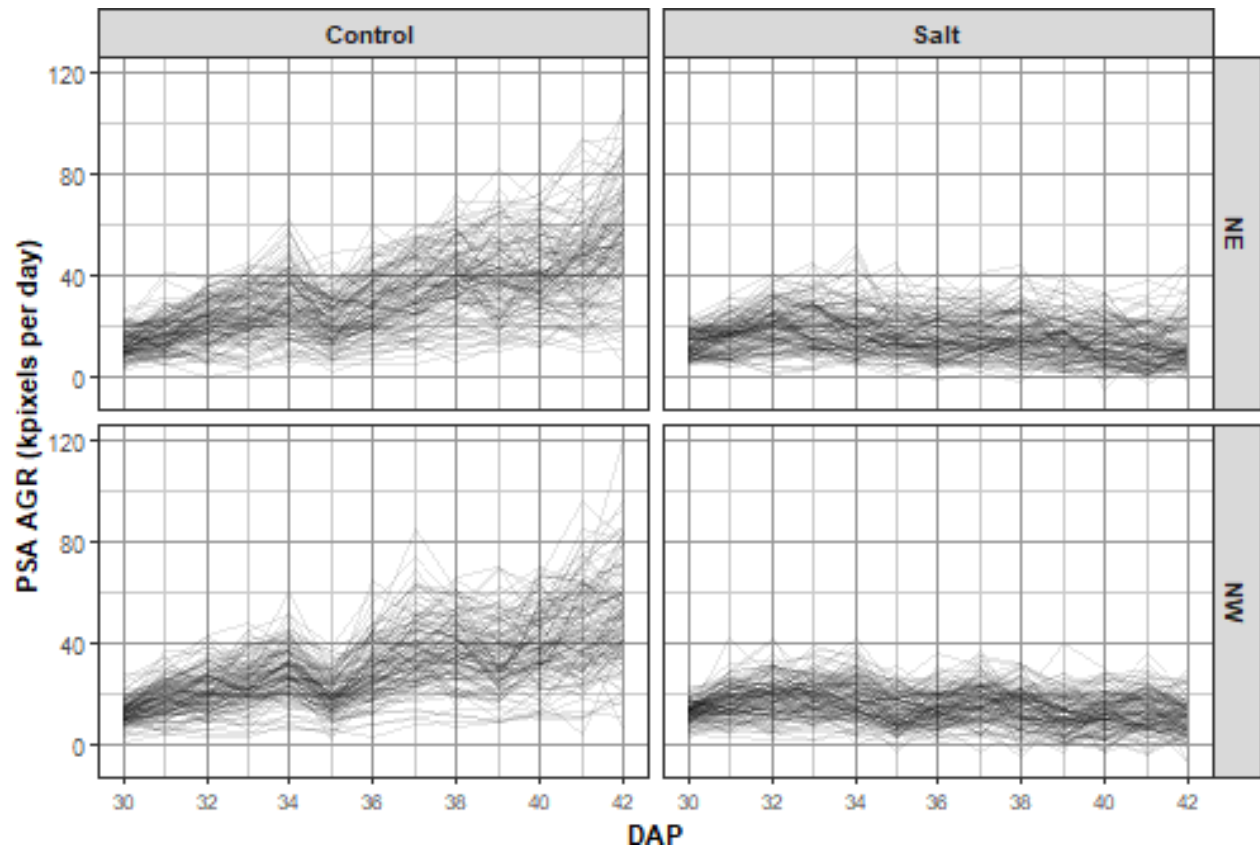
```


Plot unsmoothed profiles for all longitudinal responses

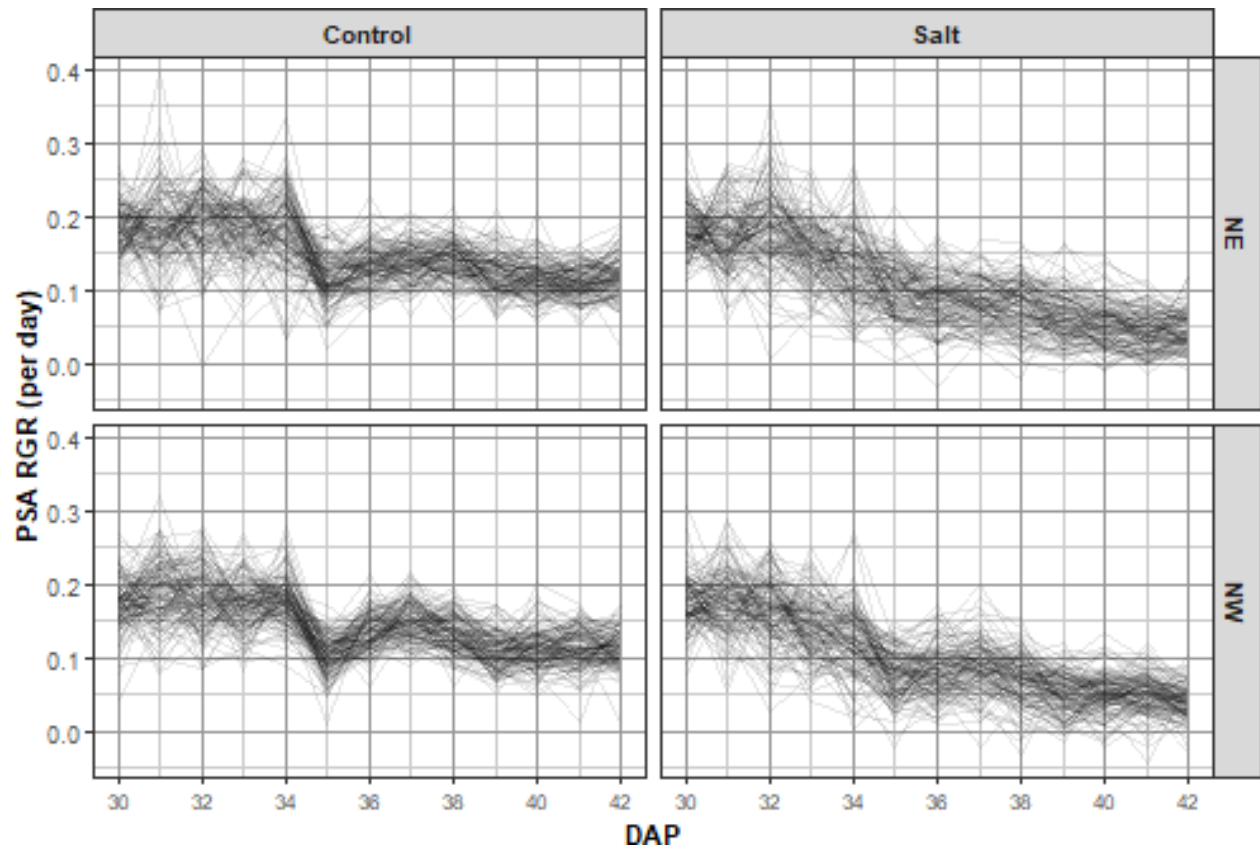
```
for (k in 1:nresp)
{
  plt <- plotProfiles(data = longi.dat, response = responses.longi[k],
    y.title = titles[k], times = "DAP",
    facet.x = "Treatment.1", facet.y = "Smarthouse",
    breaks.spacing.x = 2,
    printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1)
  print(plt)
}
```



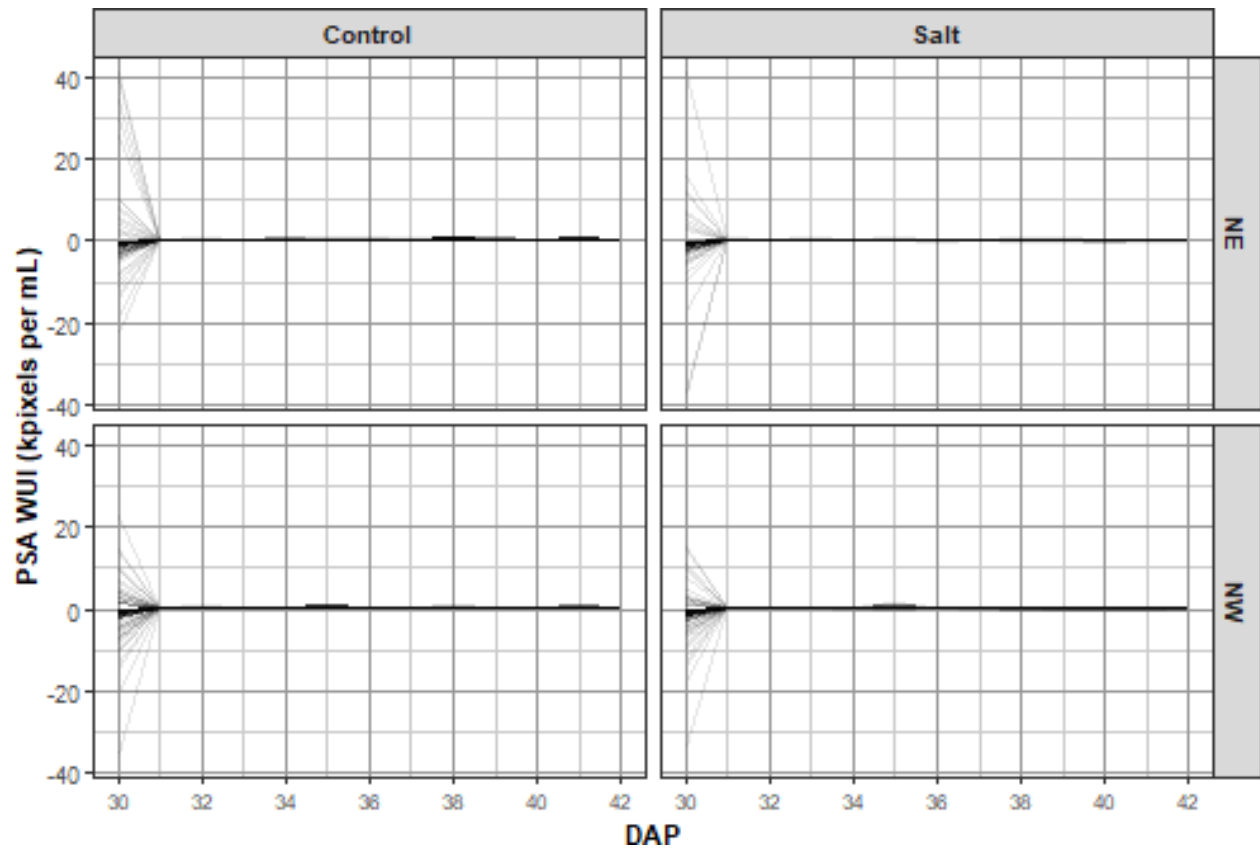
```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_vline()').
```



```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_vline()').
```

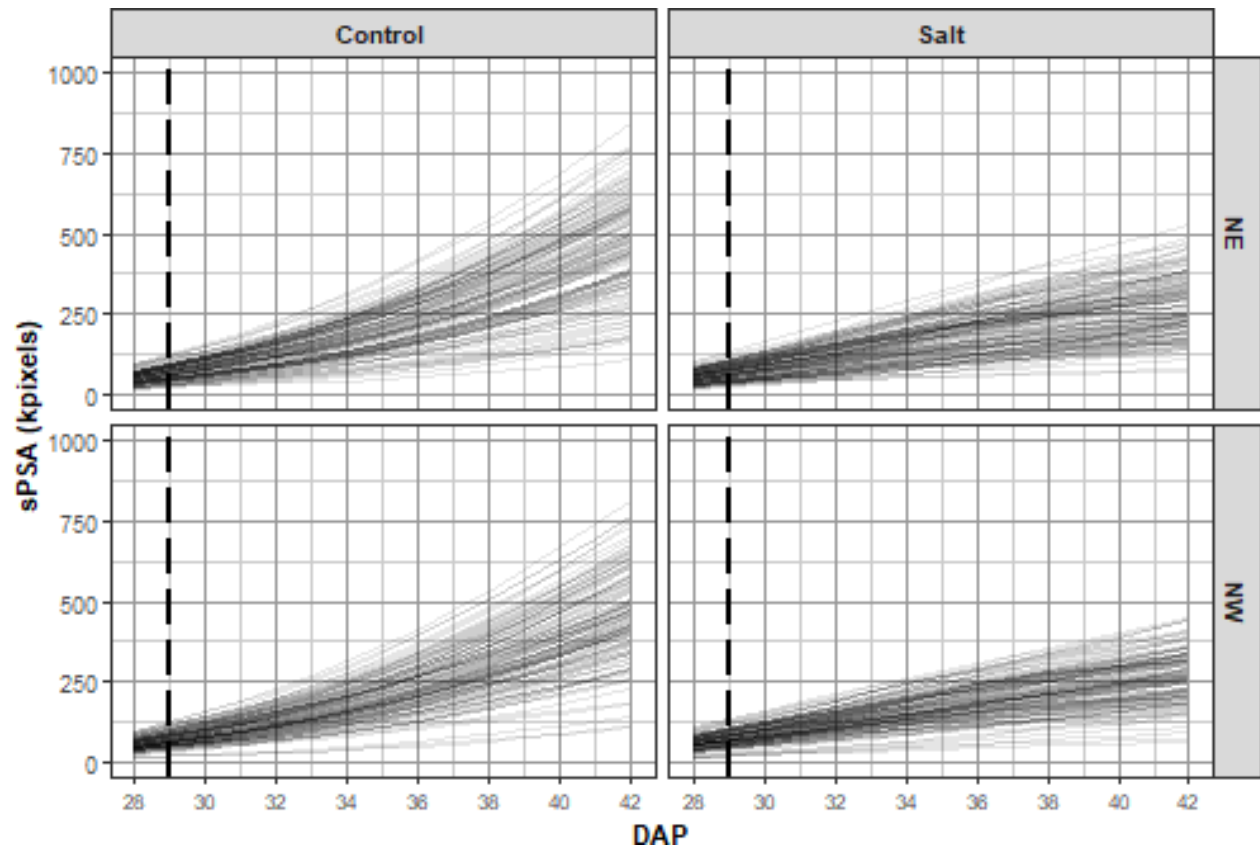


```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_vline()').
```

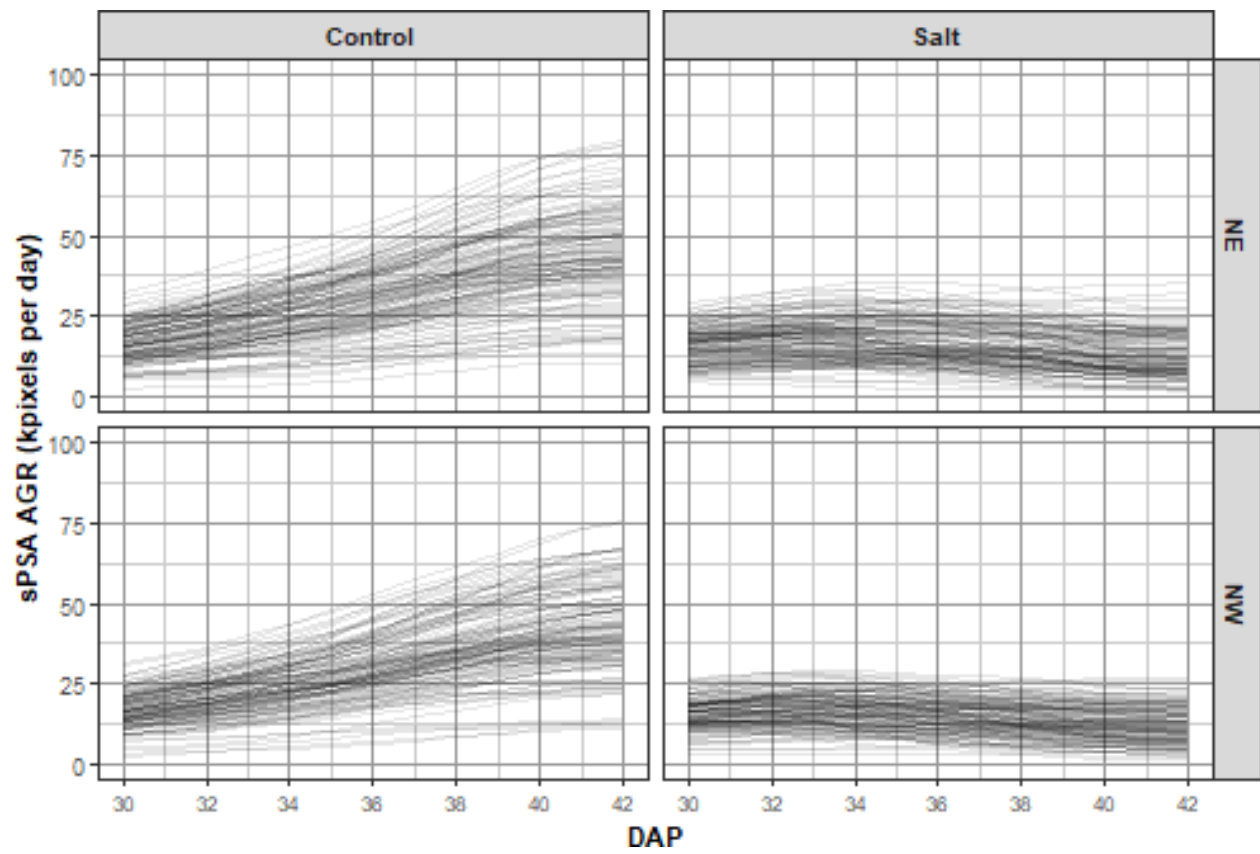


Plot smoothed profiles for all longitudinal responses

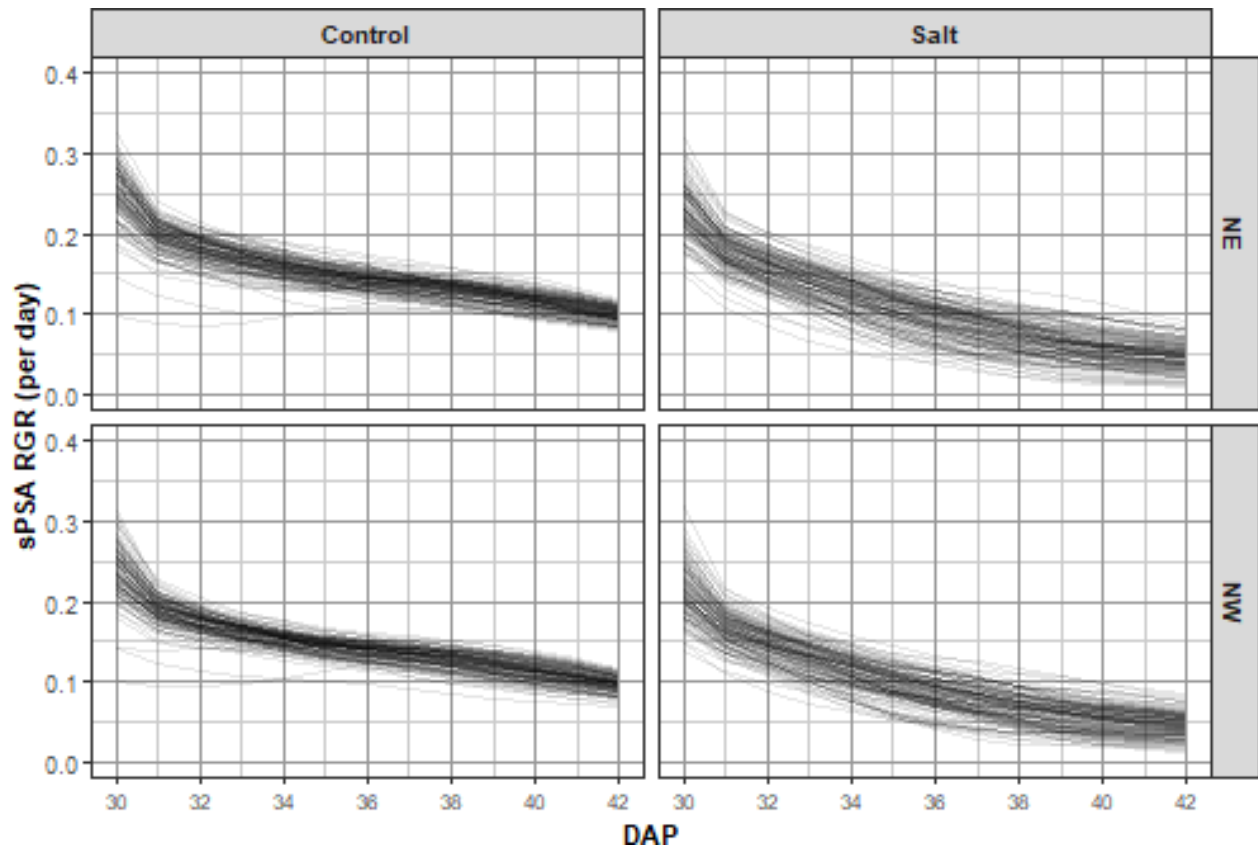
```
nresp.smooth <- length(responses.smooth.plot)
limits <- list(c(0,1000), c(0,100), c(0.0,0.40))
for (k in 1:nresp.smooth)
{
  plt <- plotProfiles(data = longi.dat, response = responses.smooth.plot[k],
    y.title = titles.smooth[k], times = "DAP",
    facet.x = "Treatment.1", facet.y = "Smarthouse",
    breaks.spacing.x = 2,
    printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1) +
    scale_y_continuous(limits=limits[[k]])
  print(plt)
}
```

```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_vline()').
```



```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_vline()').
```



Step 3: Choose the smoothing method and DF

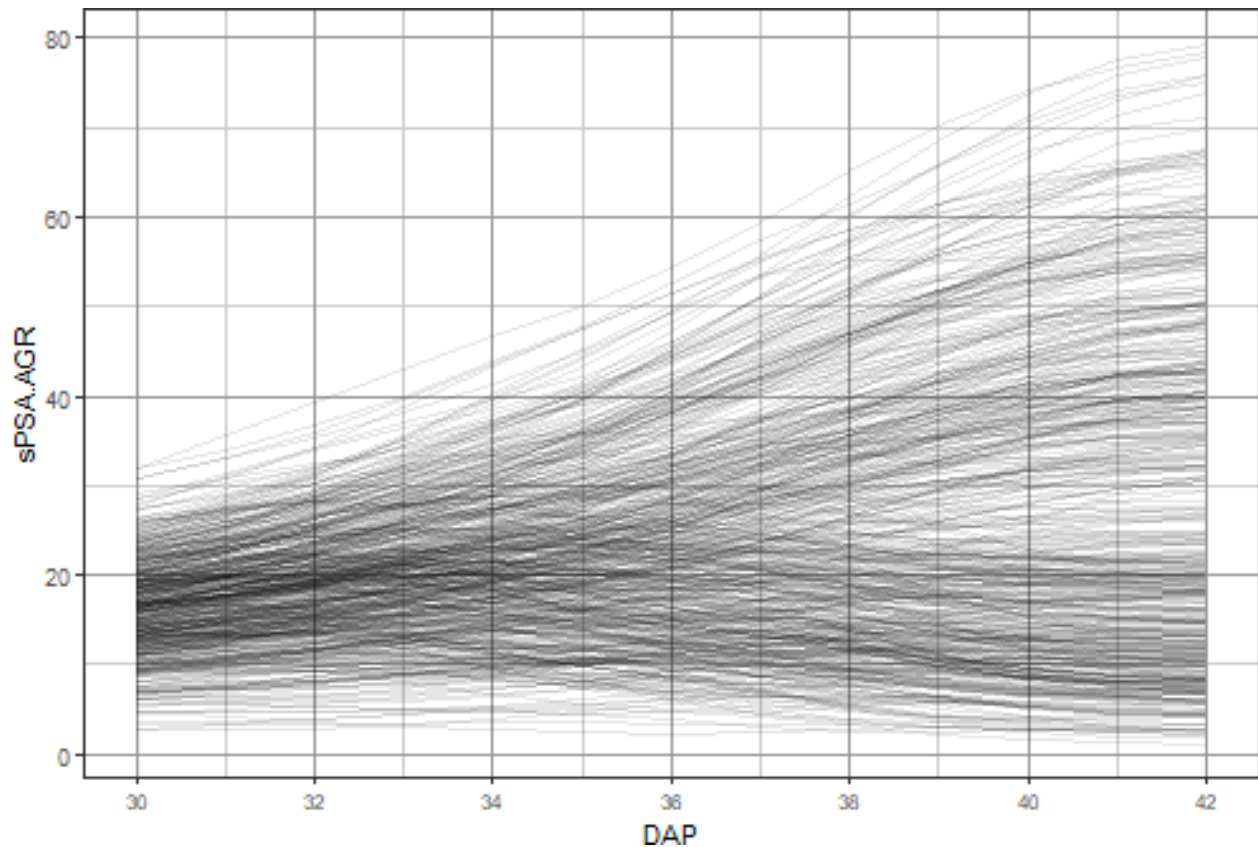
This step has been omitted.

Step 4: Identify potential outliers and clean the data

It has been decided that plants whose smoothed AGR are less than 2.5 after Day 40 are growing so slowly as to be considered anomalous. These plants are identified using `plotAnom`. Their values on Day 42 are printed. The plants are plotted without the anomalous plants followed by a plot of just the anomalous plants. The images of these anomalous plants were examined and no particular problems were identified with them. They were retained in the data.

```
anom.ID <- vector(mode = "character", length = 0L)
response <- "sPSA.AGR"
cols.output <- c("Snapshot.ID.Tag", "Smarthouse", "Lane", "Position",
                 "Treatment.1", "Genotype.ID", "Replicate", "DAP")
anomalous <- plotAnom(longi.dat, response=response, lower=2.5, start.time=40,
                     times = "DAP", vertical.line=29, breaks.spacing.x = 2,
                     whichPrint=c("innerPlot"), y.title=response)
```

```
## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom_vline()').
```

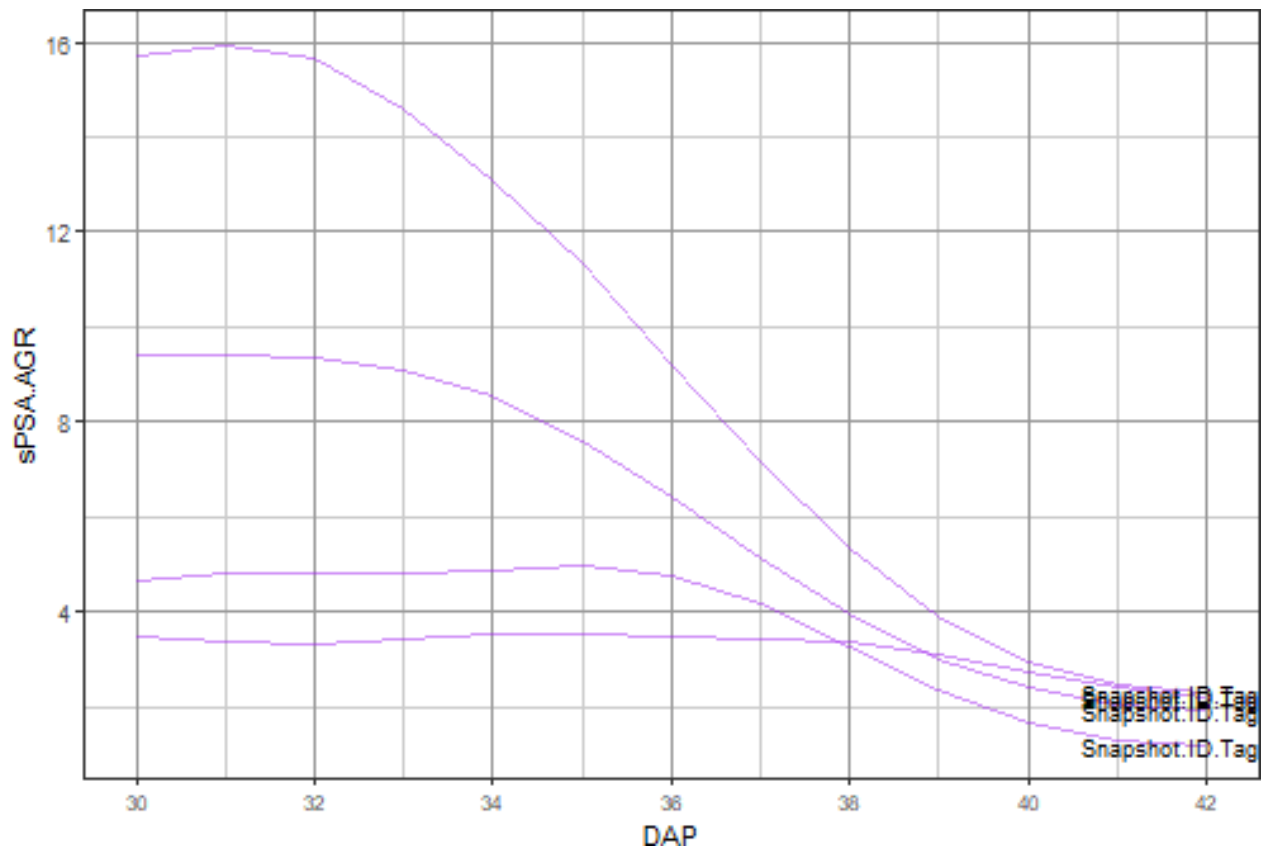


```
subs <- subset(anomalous$data, sPSA.AGR.anom & DAP==42)
if (nrow(subs) == 0)
{ cat("\n#### No anomalous data here\n\n")
} else
{
  subs <- subs[order(subs[["Smarthouse"]],subs[["Treatment.1"]], subs[[response]]),]
  print(subs[c(cols.output, response)])
  anom.ID <- unique(c(anom.ID, subs$Snapshot.ID.Tag))
  outerPlot <- anomalous$outerPlot + geom_text(data=subs,
                                                aes(x = DAP,
                                                    y = .data[[response]],
                                                    label="Snapshot.ID.Tag"),
                                                size=3, hjust=0.7, vjust=0.5)

  print(outerPlot)
}
```

```
##      Snapshot.ID.Tag Smarthouse Lane Position Treatment.1 Genotype.ID Replicate
## 1680      045575-S      NE      6      10      Salt      121701      1
## 2534      045639-S      NE      9      6      Salt      122000      1
## 5586      046144-S      NW      7      5      Salt      121133      1
## 3836      046013-S      NW      1     12      Salt      121852      2
##      DAP sPSA.AGR
## 1680  42 1.926575
## 2534  42 2.297119
## 5586  42 1.199223
## 3836  42 2.216099
```

```
## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom_vline()').
```



Step 5: Extract per-cart traits

A range of single-value plant responses are formed in Snapshot.ID.Tag order.

```
##### Set up intervals
```

```
DAP.endpts <- c(31,35,38,42)
DAP.starts <- c(31,35,31,38)
DAP.stops <- c(35,38,38,42)
DAP.mids <- (DAP.starts + DAP.stops)/2
suffices <- paste(DAP.starts, DAP.stops, sep = "to")
```

Step 5(a): Set up a data frame with factors only

```
indv.dat <- longi.dat[longi.dat$DAP == DAP.endpts[1],
  c("Smarthouse", "Lane", "Position", "Snapshot.ID.Tag",
    "cPosn", "cMainPosn",
    "Zone", "cZone", "SHZone", "ZLane", "ZMainunit", "Subunit",
    "Genotype.ID", "Treatment.1")]
indv.dat <- indv.dat[do.call(order, indv.dat), ]
```

Step 5(b): Get responses based on first and last date.

```
# Observation for first and last date
indv.dat <- cbind(indv.dat, getTimesSubset(data = longi.dat, responses = responses.image,
                                          times = "DAP", which.times = DAP.endpts[1],
                                          suffix = "first"))
indv.dat <- cbind(indv.dat, getTimesSubset(data = longi.dat, responses = responses.image,
                                          times = "DAP",
                                          which.times = DAP.endpts[length(DAP.endpts)],
                                          suffix = "last"))
indv.dat <- cbind(indv.dat, getTimesSubset(data = longi.dat, responses = "WUI.cum",
                                          times = "DAP",
                                          which.times = DAP.endpts[length(DAP.endpts)],
                                          suffix = "last"))

responses.smooth <- paste0("s", responses.image)
indv.dat <- cbind(indv.dat, getTimesSubset(data = longi.dat, responses = responses.smooth,
                                          times = "DAP", which.times = DAP.endpts[1],
                                          suffix = "first"))
indv.dat <- cbind(indv.dat, getTimesSubset(data = longi.dat, responses = responses.smooth,
                                          times = "DAP",
                                          which.times = DAP.endpts[length(DAP.endpts)],
                                          suffix = "last"))

# Growth rates over whole period.
(tottime <- DAP.endpts[length(DAP.endpts)] - DAP.endpts[1]) ## 11
```

```
## [1] 11
```

```
indv.dat <- within(indv.dat,
  {
    PSA.AGR.full <- (PSA.last - PSA.first)/tottime
    PSA.RGR.full <- log(PSA.last / PSA.first)/tottime
  })

# Calculate water index over whole period
indv.dat <- merge(indv.dat,
  byIndv4Intvl_WaterUse(data = longi.dat,
    water.use = "WU", response = "PSA",
    trait.types = c("WUI", "WUR", "WU"),
    times = "DAP",
    start.time = DAP.endpts[1],
    end.time = DAP.endpts[length(DAP.endpts)]),
  by = c("Snapshot.ID.Tag"))
```

Step 5(c): Add growth rates and water indices for intervals

```
# Growth rates for specific intervals from the smoothed data by differencing
for (r in responses.smooth)
{
  for (k in 1:length(suffixes))
```

```

{
  indiv.dat <- merge(indv.dat,
    byIndv4Intvl_GRsDiff(data = longi.dat, responses = r,
      times = "DAP",
      which.rates = c("AGR", "RGR"),
      start.time = DAP.starts[k],
      end.time = DAP.stops[k],
      suffix.interval = suffices[k]),
    by = "Snapshot.ID.Tag")
}
}

# Water indices for specific intervals from the unsmoothed and smoothed data
for (k in 1:length(suffices))
{
  indiv.dat <- merge(indv.dat,
    byIndv4Intvl_WaterUse(data = longi.dat,
      water.use = "WU", responses = "PSA",
      times = "DAP",
      trait.types = c("WU", "WUR", "WUI"),
      start.time = DAP.starts[k],
      end.time = DAP.stops[k],
      suffix.interval = suffices[k]),
    by = "Snapshot.ID.Tag")
}

indv.dat <- with(indv.dat, indiv.dat[order(Snapshot.ID.Tag), ])

```

Form continuous and interval SIITs

This experiment involved the extra step of calculating a measure of shoot ion-independent tolerance (SIIT) of pairs of plants, control and a salt-treated co-located plants.

Calculate continuous values

```

cols.retained <- c("Snapshot.ID.Tag", "Smarthouse", "Lane", "Position",
  "DAP", "Snapshot.Time.Stamp", "Hour", "xDAP",
  "Zone", "cZone", "SHZone", "ZLane", "ZMainunit",
  "cMainPosn", "Genotype.ID")
responses.GR <- c("sPSA.AGR", "sPSA.AGR", "sPSA.RGR")
suffices.results <- c("diff", "SIIT", "SIIT")
responses.SIIT <- unlist(Map(paste, responses.GR, suffices.results, sep="."))

longi.SIIT.dat <-
  twoLevelOcreate(data = longi.dat, responses = responses.GR, suffices.treatment=c("C", "S"),
    operations = c("-", "/", "/"), suffices.results = suffices.results,
    columns.retained = cols.retained,
    by = c("Smarthouse", "Zone", "ZMainunit", "DAP"))
longi.SIIT.dat <- with(longi.SIIT.dat,
  longi.SIIT.dat[order(Smarthouse, Zone, ZMainunit, DAP), ])

```

```

# Plot SIIT profiles
k <- 2
nresp <- length(responses.SIIT)
limits <- with(longi.SIIT.dat, list(c(min(sPSA.AGR.diff, na.rm=TRUE),
                                     max(sPSA.AGR.diff, na.rm=TRUE)),
                                   c(0,3),
                                   c(0,1.5)))

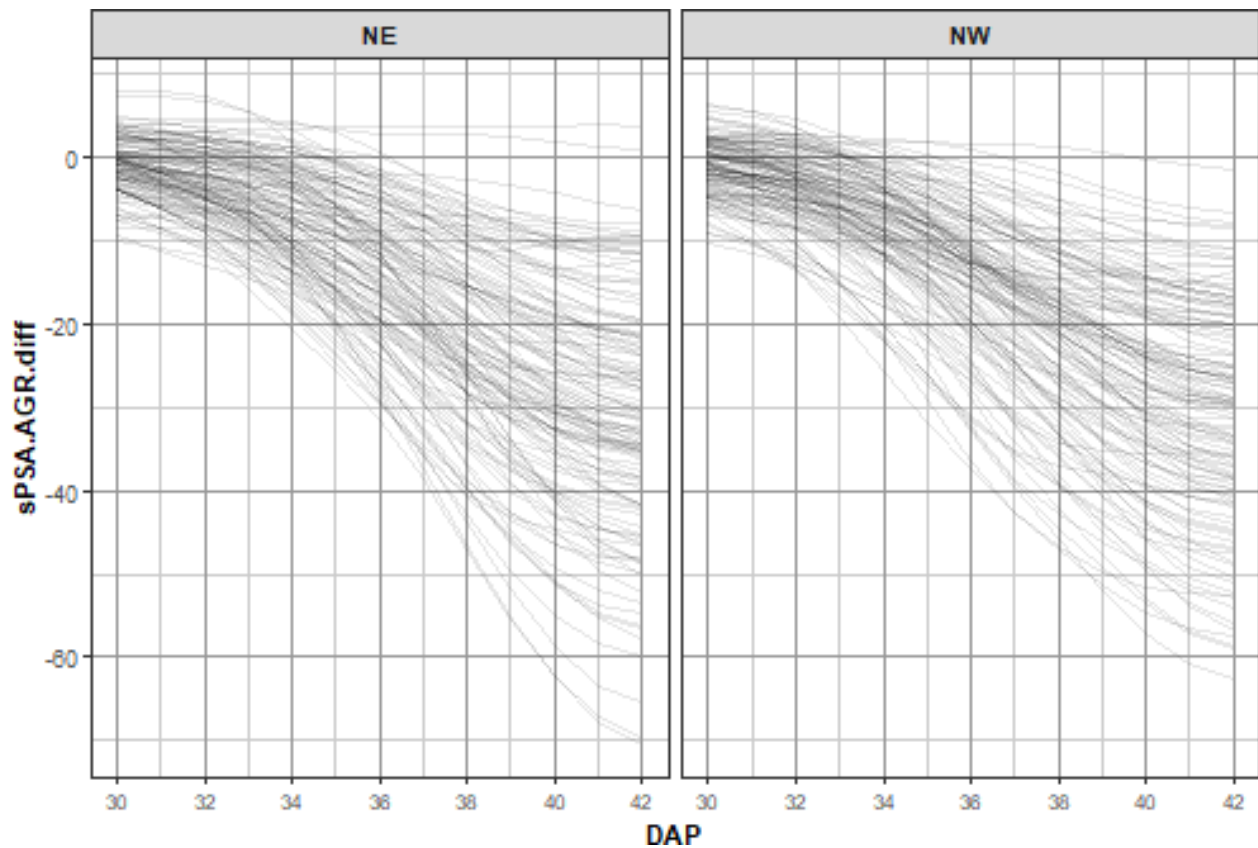
#Plots
for (k in 1:nresp)
{
  plt <- plotProfiles(data = longi.SIIT.dat, times = "DAP",
                      response = responses.SIIT[k],
                      y.title=responses.SIIT[k],
                      facet.x="Smarthouse", facet.y=".",
                      breaks.spacing.x = 2, printPlot=FALSE, )
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1) +
    scale_y_continuous(limits=limits[[k]])
  print(plt)
}

```

```

## Warning: Removed 2 rows containing missing values or values outside the scale range
## ('geom_vline()').

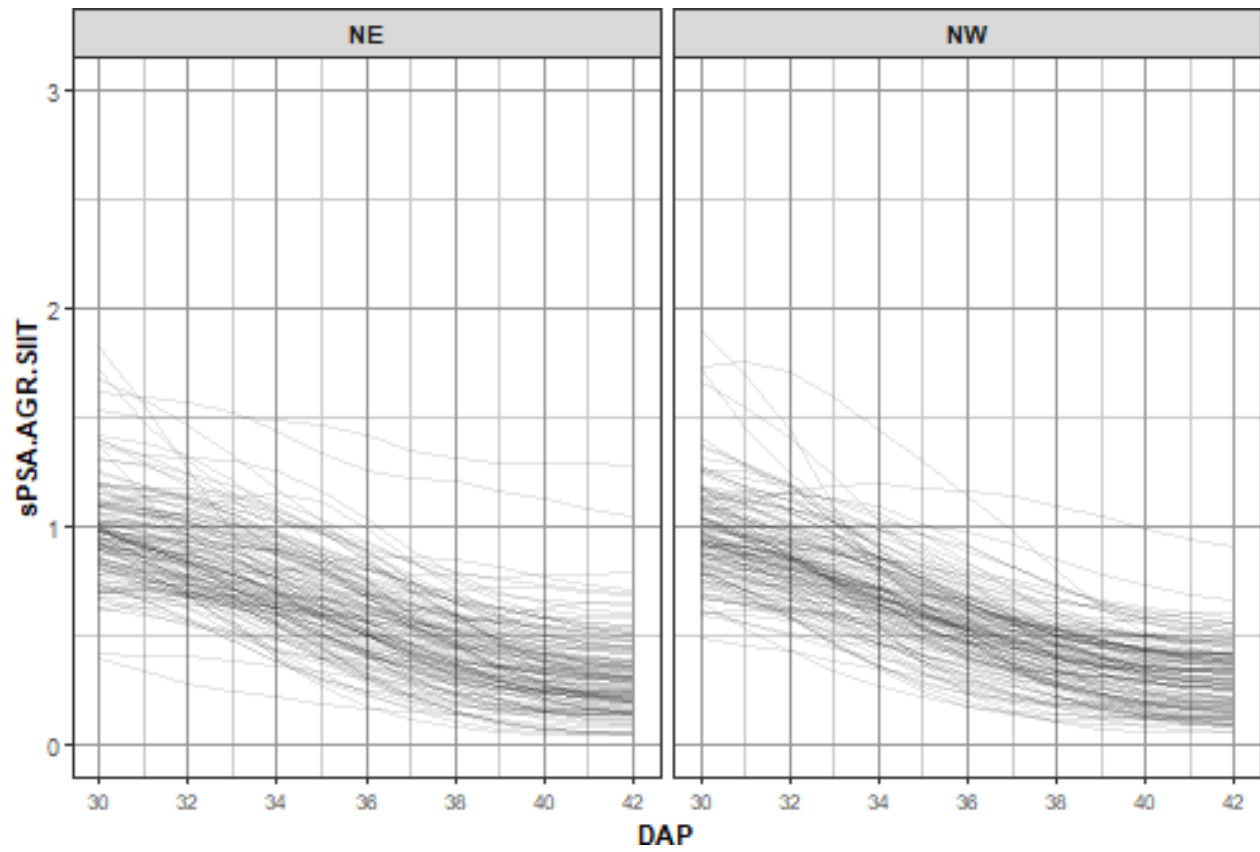
```



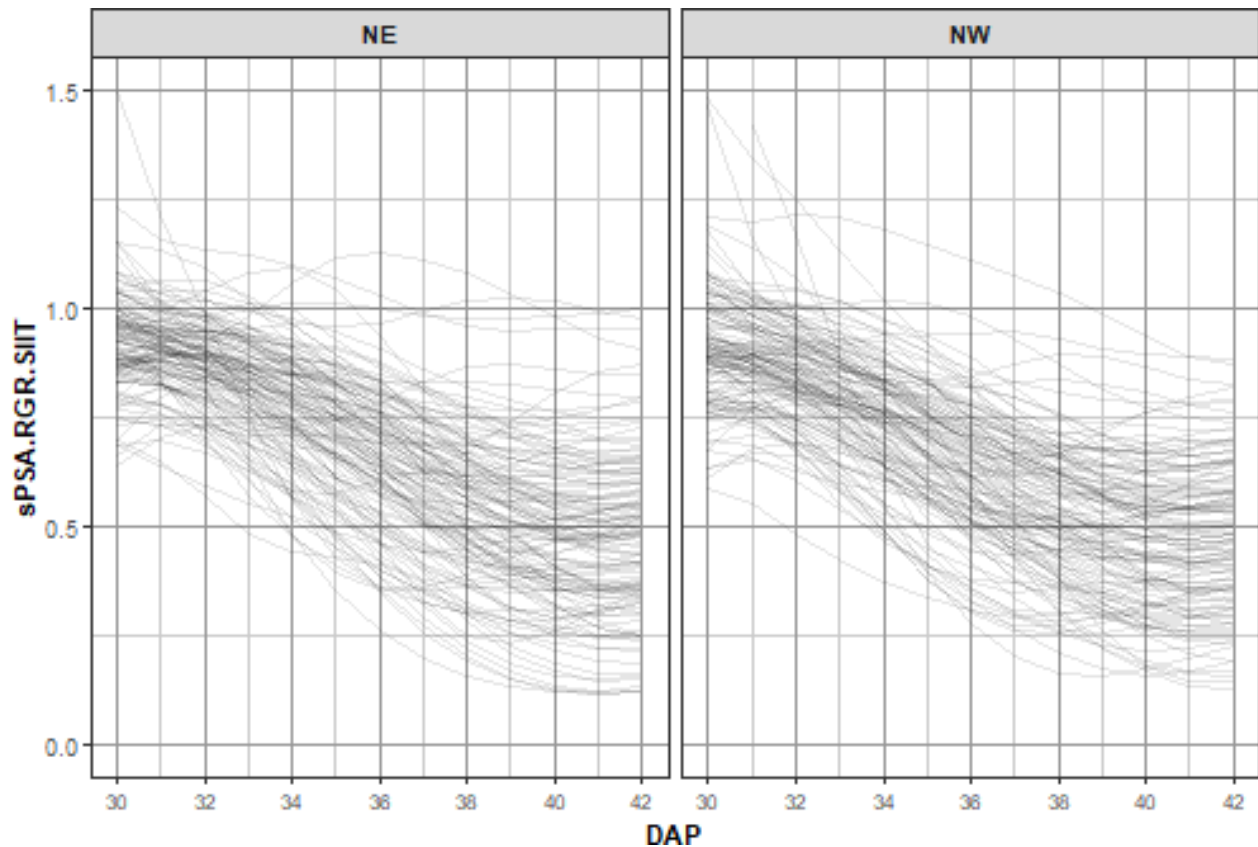
```

## Warning: Removed 2 rows containing missing values or values outside the scale range
## ('geom_vline()').

```

```
## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom_line()').
## Removed 2 rows containing missing values or values outside the scale range
## ('geom_vline()').
```



Calculate interval SIITs and check for large values for SIIT for Days 31to35

```
response <- "sPSA.RGR.31to35"
SIIT <- paste(response, "SIIT", sep=".")
responses.SIITinterval <- as.vector(outer("sPSA.RGR", suffices, paste, sep="."))

indv.SIIT.dat <- twoLevelOpcreate(data = indv.dat, responses = responses.SIITinterval,
                                suffices.treatment=c("C","S"),
                                suffices.results="SIIT",
                                columns.suffixed="Snapshot.ID.Tag")

tmp<-na.omit(indv.SIIT.dat)
print(summary(tmp[SIIT]))
```

```
## sPSA.RGR.31to35.SIIT
## Min. :0.4077
## 1st Qu.:0.7120
## Median :0.7961
## Mean :0.7876
## 3rd Qu.:0.8663
## Max. :1.1885
```

```
big.SIIT <- with(tmp, tmp[tmp[SIIT] > 1.15, c("Snapshot.ID.Tag.C", "Genotype.ID",
                                             paste(response, "C", sep=".")),
```

```

paste(response,"S",sep="."), SIIT)])
if (nrow(big.SIIT) > 1)
  big.SIIT <- big.SIIT[order(big.SIIT[SIIT]),]
print(big.SIIT)

```

```

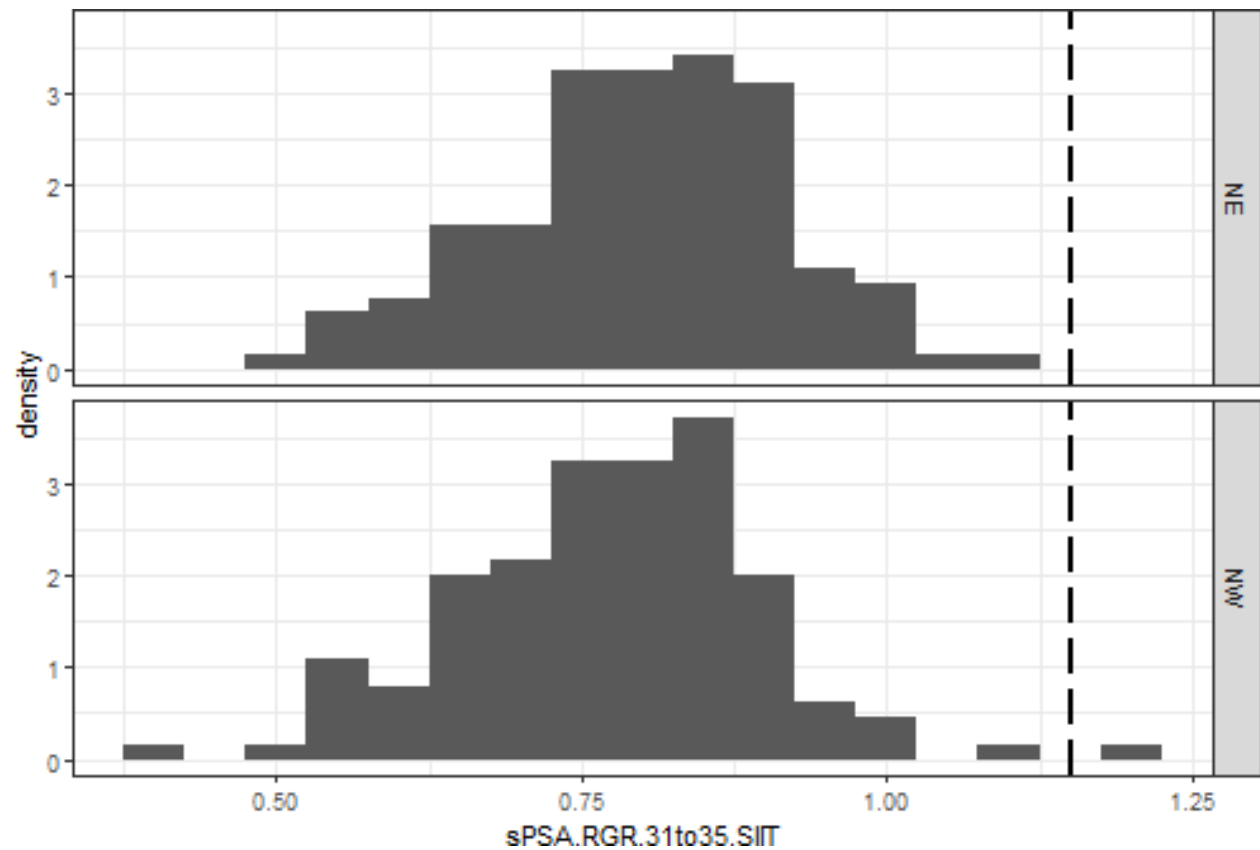
##      Snapshot.ID.Tag.C Genotype.ID sPSA.RGR.31to35.C sPSA.RGR.31to35.S
## 193      046129-C      122090      0.1310631      0.1557642
##      sPSA.RGR.31to35.SIIT
## 193      1.188467

```

```

plt <- ggplot(tmp, aes(.data[[SIIT]])) +
  geom_histogram(aes(y = after_stat(density)), binwidth=0.05) +
  geom_vline(xintercept=1.15, linetype="longdash", linewidth=1) +
  theme_bw() + facet_grid(Smarthouse ~.)
print(plt)

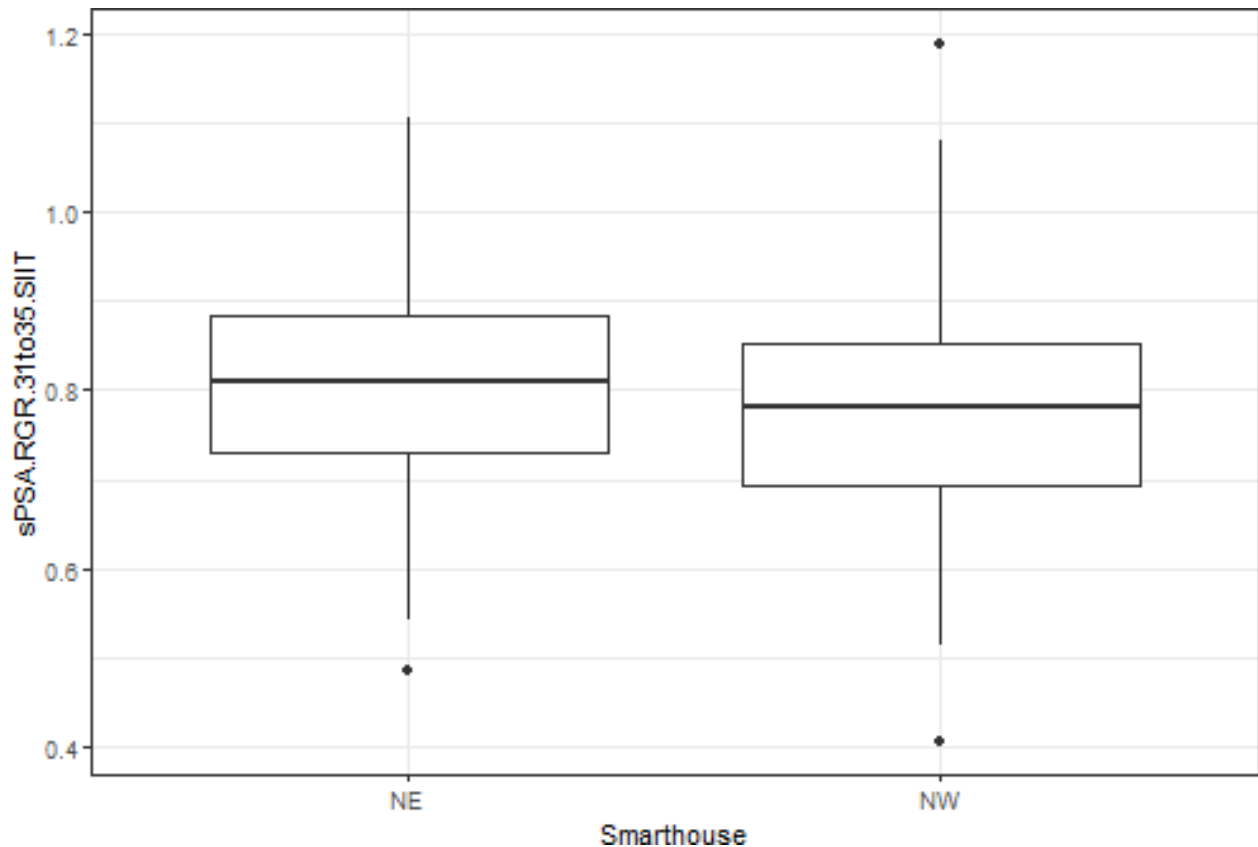
```



```

plt <- ggplot(tmp, aes(x=Smarthouse, y=.data[[SIIT]])) +
  geom_boxplot() + theme_bw()
print(plt)

```



```
remove(tmp)
```

Save image

```
save.image("Rice.RData")
```

References

Al-Tamimi, N, Brien, C.J., Oakey, H., Berger, B., Saade, S., Ho, Y. S., Schmockel, S. M., Tester, M. and Negrao, S. (2016) New salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nature Communications*, **7**, 13342.

Brien, C. J. (2026g) `growthPheno`: *Functional Analysis of Phenotypic Growth Data to Smooth and Extract Traits*. Version 3.1.20. <https://cran.r-project.org/package=growthPheno>.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J. and Thompson, R. (2023). *ASReml-R Reference Manual Version 4.2*. VSN International Ltd, <https://asreml.kb.vsni.co.uk/>.

Brien, C., Jewell, N., Garnett, T., Watts-Williams, S. J., & Berger, B. (2020). Smoothing and extraction of traits in the growth analysis of noninvasive phenotypic data. *Plant Methods*, **16**, 36. <http://dx.doi.org/10.1186/s13007-020-00577-6>.

R Core Team (2026) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.r-project.org/>.